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MicroRNAs: Novel Regulators During the Immune Response

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MicroRNAs (miRNAs), an abundant class of highly conversed, small noncoding RNAs, present an entirely new way of post-transcriptional gene regulation. miRNAs play a key role in diverse biological processes, such as embryogenesis, differentiation, inflammation, viral infections, and carcinogenesis. Recently, more studies showed the importance of these noncoding small RNAs on immune system development and response, and miRNAs are found to involve in the regulation of immunity, including the development and differentiation of immnue cells, antibody production and the inflammatory mediator release. Here, the latest findings were summarized to explore the function and mechanism of miRNAs in modulating innate and adaptive immune responses.

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MicroRNAs are small (~22 nt), noncoding, double-stranded RNA molecules, and they can regulate gene expression which function primarily by reducing the abilities of specific mRNAs to direct the synthesis of their encoded proteins. The first recognized miRNA found in 1993 is *lin-4*, which was genetically identified as heterochronic genes controlling the developmental timing of cell fate at larval stages in *Caenorhabditis elegans* (Lee et al., 1993; Wightman et al., 1993). Since the initial observation, more than 700 miRNAs have been identified in mammalian cells and have been shown to play important roles in development, differentiation and homeostasis (Stefani and Slack, 2008; Williams, 2008). Bioinformatic approaches further suggested that the mammalian miRNA repertoire might collectively regulate several thousand genes (Lewis et al., 2005), and 30% of all protein-encoding genes are estimated to be regulated by them (Bartel, 2004). Since miRNAs act as key regulators in a wide variety of biological processes, it is now apparent that abnormal miRNA expression is a common feature of various diseases, notably cancer (Lu et al., 2005; Negrini et al., 2007; Osada and Takahashi, 2007; Sassen et al., 2008). Recently, more miRNAs are reported to involve in regulation of immune systems, demonstrating that miRNAs modulate many aspects of the immune responses such as differentiation, survival and function of immune cells, the cytokine responses as well as the intracellular signaling pathways. In this review, we try to summarize the recent evidence and focus on the regulation function of miRNA during the immune responses.

miRNAs Biogenesis and Functions

Human miRNAs are present in introns of coding genes and introns and exons of noncoding transcripts (Rodriguez et al., 2004). To generate mature miRNAs, primary miRNAs (pri-miRNAs) from long primary transcripts go through a series of endonucleolytic steps (Fig. 1). Pri-miRNAs containing 5′ 7-methylguanosine cap and 3′ poly (A) tail are transcribed by RNA polymerase II (Cai et al., 2004; Lee et al., 2004), where others by RNA polymerase III (Borchert et al., 2006). The pri-miRNAs are then recognized and cleaved by the Drosha-DGCR8 microprocessor complex (Denli et al., 2004; Gregory et al., 2004; Han et al., 2004; Landthaler et al., 2004), to a ~70 nt intermediate with the typical stem-loop hairpin structure, precursor miRNAs (pre-miRNAs), in the nucleus. After transported to the cytoplasm by the RanGTP-dependent dsRNA-binding protein Exportin 5 (Bohnsack et al., 2004; Lund et al., 2004), the pre-miRNAs are further processed into ~22 nt double-stranded miRNA duplex by the cytoplasmic RNase III enzyme Dicer (Ketting et al., 2001). One strand of this miRNA duplex, which is destined as the guide strand, incorporates into a large protein complex, RNA-induced silencing complex (RISC) which was formed by Dicer, TRBP (a dsRNA-binding domain protein) and Ago2 (the Argonaute protein 2), and finally becomes the mature miRNA. At the same time, the other strand, the so-called passenger strand, is degraded.

Each mature miRNA interacts with a specific mRNA, typically through pairing of nucleotide bases between the miRNA sequence and complementary sequences in the mRNA's 3′-untranslated region (3′UTR). As gene regulators, the functions of miRNAs are mediated through translational repression or mRNA degradation. In general, whether target mRNAs are cleaved and degraded is mainly determined by the complementarity between miRNAs and target mRNAs (Bartel, 2004; Engels and Hutvagner, 2006). Perfect sequence complementarity leads to endonucleolytic cleavage and degradation of the mRNA, which is more suitable for plant miRNAs, that have perfect sequence complementarity. Whereas less strict complementarity results in translational

Abbreviations: BCR, B cell receptor; DC, dendritic cell; DN, double-negative T cell; DP, double-positive T cell; MΦ, macrophage; TCR, T cell receptor; Th, help T cell; TLR, toll-like receptor; TNF-α, tumor necrosis factor α; T reg, regulatory T cell.

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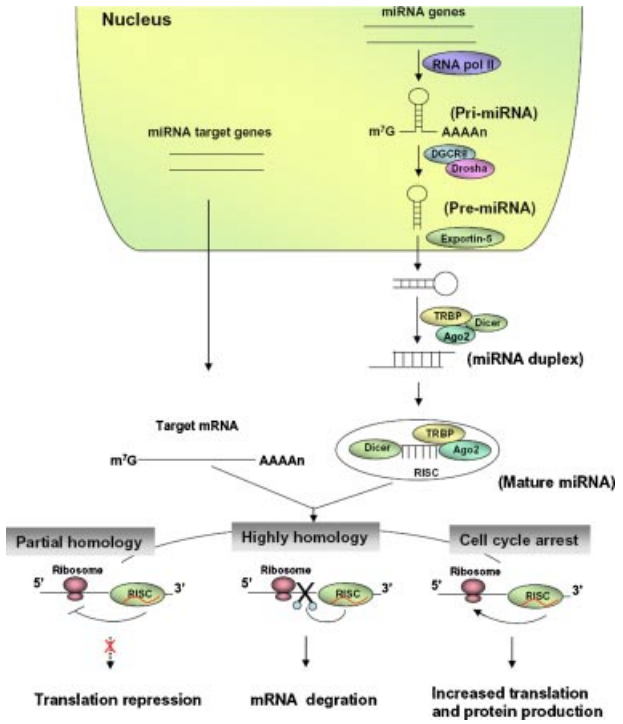

Fig. 1. The biogenesis and function of miRNAs. Primary miRNA (Pri-miRNA) is transcribed by RNA polymerase II or polymerase III, and then is recognized and cleaved by the Drosha-DGCR8 microprocessor complex to precursor miRNA(pre-miRNA). After export into the cytoplasm by protein Exportin 5, the pre-miRNA is further processed into ~22 nt double-stranded miRNA duplex by the cytoplasmic RNase III enzyme Dicer. One strand of this miRNA duplex incorporates into a large protein complex, RNA-induced silencing complex (RISC), and finally becomes the mature miRNA. Meanwhile, the complementary strand is usually rapidly degraded. The mature miRNA can bind to the target messenger RNA by base pairing, causing inhibition of protein translation or degradation of the target messenger RNA according to the degree of homology between miRNA and target RNA. Notably, in the situation of cell cycle arrest certain miRNA can also enhance mRNA translation although they repress translation elsewhere in the cell cycle. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

repression. In animal cells, the imperfect match between miRNA and target RNA often preclude the endonucleolytic cleavage of mRNA and lead to translation repression. Although most studies focus on the miRNA-mediated gene silencing, Vasudevan et al. (2007) showed that miRNA can also increase translation. In their studies, a specific miRNA (miR369-3), the well-studied "repressive" *let7* miRNA and the artificial miRNA mimic *cxcr* can also enhance mRNA translation during starvation-induced G1 arrest, whereas they repress translation elsewhere in the cell cycle (Vasudevan et al., 2007). This research broadens the effect of miRNAs on protein expression, and also increases the complication of miRNAs applications.

miRNAs During the Innate Immune Responses

miRNAs have an important role in modulating innate immune responses, the first line of defense to bacterial, viral, and other pathogens. During inflammatory response, several hundred genes are involved and a process to achieve pathogen clearance and at the same time avoid consequences of dysregulated gene expression must be tightly regulated. Recent studies have shown several miRNAs, such as miR-155, miR-146, and miR-223 etc. (Fig. 2), regulate the acute inflammatory response after the recognition of pathogens by the Toll-like receptors (TLRs) (Taganov et al., 2006; O'Connell et al., 2007; Tili et al., 2007; Perry et al., 2008).

miRNA and Macrophage/Monocytes

To examine the potential involvement of miRNAs in regulation of the innate immune response, Taganov et al. (2006) analyzed expression of 200 miRNAs after exposure of human monocytic THP-1 cell line to LPS. They showed three miRNAs were up-regulated, namely miR-146, miR-132, and miR-155. Further, induction of miR-146 by the TLR system displayed dual occurrence: TLRs that recognize bacterial constituents and reside on the cell surface (like TLR2, TLR4, and TLR5) trigger miR-146 induction; those TLRs that mainly sense viral nucleic acids and localize intracellularly (TLR3, TLR7, and TLR9) have little effect on miR-146 expression. Promoter analysis of the miR-146a revealed that it is regulated by NF-κB and may function as a negative regulator of IRAK1 (IL-1 receptor-associated kinase 1) and TRAF6 (TNF receptor-associated factor 6) expression (Taganov et al., 2006). As it is well known that TRAF6 and IRAK1 are two key adapter molecules in the downstream of TLRs, which can trigger the activation of IκB kinase (IKK) and the Jun kinase (JNK), then in turn, activate NF-κB and activating protein (AP)-1 transcription factors, and finally result in up-regulation of immune-responsive genes (Liu and Zhao, 2007). In addition to TLRs stimulations, Perry's study verified miR-146a expression was related to IL-1β-induced responses (Perry et al., 2008). They demonstrated an important feedback mechanism during severe inflammation: IL-1β-induced increases in miRNA-146a expression negatively regulate IL-8 and RANTES release. Recent study found this miRNA to be associated with psoriasis, a chronic inflammatory skin disease, indicating that alterations in the fine-tuning of innate immune responses by miRNAs may contribute to inflammatory disorders (Sonkoly et al., 2007). Taken together, miR-146a now was thought to be a negative regulator during the innate immune responses; and the transcriptional regulators of miR-146b are unknown.

MiR-155, one of the first miRNAs associated with cancer, now has been demonstrated important and multi-roles during an innate immune response.

Microarray studies identified miRNAs induced in primary murine macrophages after exposure to polyriboinosinic: polyribocytidylic acid [poly (I: C)] (which binds to TLR3) or the cytokine IFN-β, and miR-155 was the only miRNA of those tested that was substantially up-regulated by both stimuli (O'Connell et al., 2007). To determine whether other TLR ligands also could induce miR-155, macrophages were stimulated with LPS, which signals through TLR4 (Medzhitov et al., 1997; Poltorak et al., 1998); hypomethylated DNA (CpG), a TLR9 ligand (Hemmi et al., 2000); or Pam3CSK4, a synthetic lipoprotein that activates TLR2 (Brightbill et al., 1999), and all four TLR ligands (including TLR3) tested induced strong expression of miR-155. In contrast to TLR ligands, IFN-β/γ requires TNF-α autocrine/paracrine signaling to up-regulate miR-155 in macrophages. Tili et al. (2007) showed the relationship between miR-155 and TNF-α which is the main cytokine produced by macrophages in response to LPS. They found the level of miR-155 in macrophage oscillated rapidly following TNF-α stimulation, which is similar to the oscillatory activity of NF-κB. This implied miR-155 might exert both positive and negative effects by acting post-transcriptionally to regulate expression of different target proteins, such as IKKε, FADD (Fas-associated death domain) and Ripk1 (receptor interacting serine-threonine kinase 1). Further, miR-155 was found to enhance the production of TNF-α, which suggested the positive role of miR-155 to regulate the release of inflammatory mediators during the innate immune response.

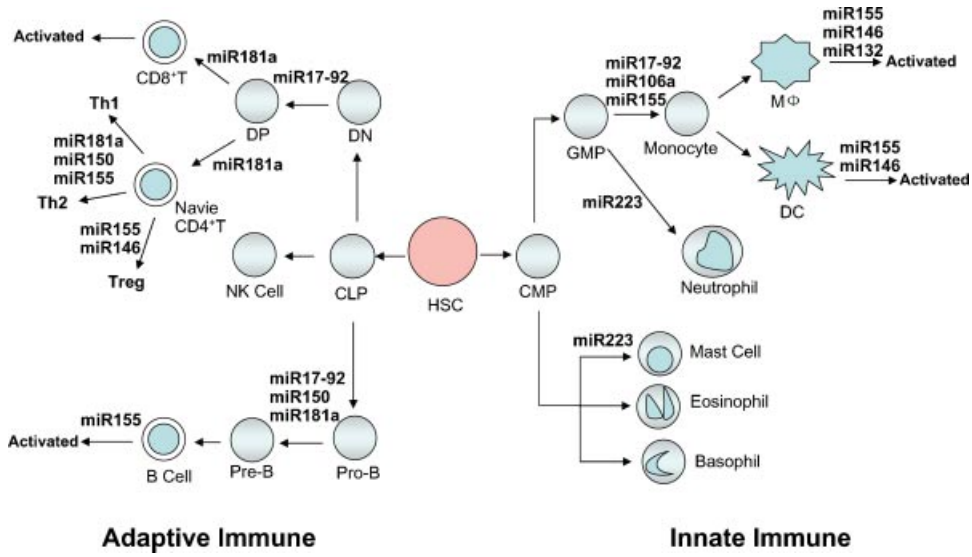

Fig. 2. Overview of major miRNAs during the adaptive and innate immune responses. HSC, hematopoietic stem cell; CLP, common lymphocyte progenitor; CMP, common myeloid progenitor; GMP, granulocyte monocytic progenitor; DN, double-negative T cell; DP, double-positive T cell; Th, help T cell; T reg, regulatory T cell; DC, dendritic cell; MΦ, macrophage. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

This hypothesis is supported by the results of an array analysis of gene expression in the *Eμ-miR-155* transgenic mice that overexpressed miR-155 in B cells, which demonstrate an elevated level of serum TNF-α (Tili et al., 2007). In addition, in miR-155 knock-out mice, miR-155 was also verified the requirement for the normal immune function (Rodriguez et al., 2007), which will be discussed in the following adaptive immune responses. All the studies suggest a crucial role of miR-155 in macrophages immune responses.

In contrast to miR-155, miR-125b acts as a post-transcriptional repressor of TNF-α transcript (Tili et al., 2007). The mechanisms of miR-125b down-regulating expression of TNF-α may be include, repression of translation by targeting its 3′-UTR, rapid deadenylation of TNF-α transcript, or both. It is hypothesized that relative high levels of miR-125b in macrophages without the presence of LPS may be essential to ensure that macrophages are not activated without microbial infection, and the down-regulation of miR-125b in response to LPS may be required for proper TNF-α production and the innate immune response (Tili et al., 2007).

Moschos et al. (2007) measured the differential mature miRNA expression profile during the innate immune response to aerosilized LPS in the mouse lung. They found 12 miRNAs (miR-21, -25, -27b, -100, 140, -142–3p, -181c, 187, -194, -214, -223, and -224), which were involved in the innate immune response, were significantly up-regulated in a time dependent fashion. Unlike the LPS-induced response in THP-1 cells and mouse macrophages (Taganov et al., 2006; O'Connell et al., 2007), expression of miRNA-146 or -155 were not found to upregulate. They speculated the reasons behind these differences could be a result of multiple factors including the presence of multiple cell types within the lung or be related to the dynamics of the two models.

miRNA and Granulocyte

miRNA-223 was found to have crucial roles in regulating granulocyte proliferation and activation. Its expression was bone marrow-specific and was confined to myeloid cell lineages (Chen, 2004). However, overexpression of miR-223 and knock-down of miR-223, studied by two independent groups, showed the opposite effect (Fazi et al., 2005; Johnnidis et al., 2008). Fazi et al. (2005) firstly reported that miR-223 was a positive regulator of granulocyte differentiation by both overexpression and knockdown experiments. In their study, two transcription factors named NFI-A (nuclear factor I-A) and C/EBPα (the CCAAT enhancer proteins), had been implicated to regulate miR-223 expression. These two factors compete for binding to the miR-223 promoter. NFI-A is required to maintain miR-223 at low levels, then during differentiation NFI-A is replaced by the transcription factor C/EBPα, which induces high expression of miR-223 (Fazi et al., 2005). This, in turn, represses the expression of NFI-A post-transcriptionally. The role of C/EBPα in regulating miR-223 expression was confirmed by Fukao et al. (2007), who demonstrated C/EBPα can combine with PU.1 (another myeloid specific transcription factor) to enhance the promoter activity. More recently, observations in miR-223 knockout mice revealed the negative role of miR-223 in regulating progenitor proliferation and granulocyte differentiation and activation (Johnnidis et al., 2008). They also found a critical target of miR-223 in early myeloid progenitors, Mef (myeloid ELF-1-like factor) 2c. That genetic ablation of Mef2c suppresses progenitor expansion and corrects the neutrophilic phenotype in miR-223 null mice, suggested that the action of miR-223 is probably mediated by downregulation of Mef2c. As for the contradiction with Fazi et al. results, they speculated several reasons such as method diversity, time specificity and different stages of myeloid cell development. Although paradoxical results exit, the role of miR-223 in regulating granulocyte production and the inflammatory responses was indispensable.

miRNAs in the Adaptive Immune Responses

In addition to regulating innate immune responses, miRNAs also have an important role in modulating adaptive immune responses (Fig. 2), a central feature of which was activation and subsequent clonal expansion of antigenspecific lymphocytes.

miRNA and T Cell Differentiation and Activation

Dynamic regulation of miRNA expression in several distinct stages of T cell was observed by Neilson et al. (2007). They found the degree of miRNA variation, whether quantity or content, across the T-lymphocyte developmental progression is striking, suggesting global miRNA levels are correlated to the level of T cell differentiation, which miRNAs take part in the regulation of T cell differentiation and activation? Recently, the miRNA expression profile in antigen-specific naïve, effector and memory CD8 T cells was analyzed using three different methods (Wu et al., 2007). Among miRNAs, expressed in all the T cell subsets, the frequency of seven miRNAs (miR-16, miR-21, miR-142-3p, miR-142-5p, miR-150, miR-15b, and let-7f) alone accounted for 60% of all miRNAs. Compared with naïve cells, global downregulation of miRNAs (including six out of the seven dominantly expressed miRNAs) was observed in effector T cells, and in memory T cells the miRNA expression levels tended to come back up though they were still lower than in naïve cells. It seemed that the level of most miRNAs expression appear to inversely correlate with the activation status of the cells, exception of miR-21. Since regulation of miRNA expression was found to characterize the stage-specific development of thymocytes (Neilson et al., 2007), miRNAs may modulate the differentiation status of antigen-stimulated T cells.

As stated above, different development stages and cell types of T cells have distinct miRNA expression profiles. However, studies on the relationship between the expression and functions of these miRNAs are limited. Li et al. (2007) demonstrate that miR-181a can influence T cell sensitivity to antigens by modulating T cell receptors (TCRs) signaling strength. T cell sensitivity, achieved by TCR, is intrinsically regulated during maturation to ensure proper development of immunity and tolerance. The expression of miR-181a is higher in immature T cell populations such as DP thymocytes, but lower in the more differentiated T cell populations such as Th1 and Th2 effector cells. The inhibition of this miRNA significantly impairs DP cell sensitivity and efficiently obstructs positive and negative selection, whereas increasingly miR-181 expression in mature T cells augments their sensitivity to peptide antigens (Li et al., 2007). Moreover, quantitative regulation of T cell sensitivity by miR-181a enables mature T cells to recognize antagonists-the inhibitory peptide antigens-as agonists. Mechanism study demonstrated that this did not result from changes in the expression of surface receptors but instead involved the coordinated downregulation of multiple phosphatases. Multi-target regulation by miR-181a is required for fine-tuning T cell sensitivity, thus, miR-181a was thought an intrinsic modulator of T cell sensitivity and selection (Li et al., 2007). In addition, another research also proved miR-181a was involved in positive selection of T cell through repressing the expression of BCL-2, CD69, and the T cell receptor (Neilson et al., 2007). Although former study showed ectopically expressed miR-181 in mice Lin^- bone marrow cells modestly decrease $CD8^+$T cells but almost have no effects on $CD4^+$T cells in vivo (Chen et al., 2004), miR-181a is critical in T cell development and regulates the quantitative levels of the T cell response to antigens. Recently, the study aimed to find miRNA target genes related to immune systems showed that miR-181a targeted ~600 genes (single algorithm prediction—PicTar) which included 10 phosphatases but also other factors that could influence T cell function, including *CD4*, *BCL6*, *MECP2*, and *TGFBR1* (Asirvatham et al., 2008).

In addition to miR-181a, miR-155 is also involved in T cell differentiation and activation. In miR-155 null mice, $CD4^+$T cells are intrinsically biased toward Th2 differentiation compared with Th1 cells, with enhanced levels of the Th2 cytokines IL-4, IL-5, and IL-10 (Rodriguez et al., 2007; Vigorito et al., 2007). Further, a transcription factor *c-Maf* was identified to contribute to the increased Th2 cytokine production (Rodriguez et al., 2007). Therefore, miR-155 modulates level of *c-Maf* in T cells and this is likely to induce the attenuation of Th2 cell responses in vivo.

Regulatory CD4 T (T reg) cells are essential for immune regulation (Brunkow et al., 2001; Fontenot and Rudensky, 2005). Cobb et al. (2006) have shown a miRNA profile of T reg, which is different from naive CD4 T cells, but similar to that of acutely actived CD4 T cells. Although the extensive overlap between the miRNA profile of T reg cells and activated T cells, they also found miRNAs that are overexpressed by T reg cells but not by activated T cells, for example miR-223 and miR-146. In addition, they identified the importance of Dicer for the development of natural T reg cells in the thymus and the efficient induction of Foxp3. More recently, to test the role of miRNAs in the development and function of T reg cells, Zhou et al. (2008) inactivate miRNA generation selectively in T reg cells by crossing a FoxP3-GFP-hCre transgenic mouse to a conditional Dicer knockout mouse. Phenotypic analysis supports a central role for miRNAs in maintaining the stability of differentiated T reg cell function in vivo and homeostasis of the adaptive immune system. In the same issue of JEM, other two groups also reported their research respectively to support the view that miRNA-dependent regulation is critical for preventing spontaneous inflammation and autoimmunity (Chong et al., 2008; Liston et al., 2008).

miRNA and B Cell

During B cell differentiation, miR-150, which is specifically expressed by mature lymphocytes, was found to play a key role (Monticelli et al., 2005; Xiao et al., 2007; Zhou et al., 2007). High level expression of miR-150 in the spleen and the thymus suggested that it may participate in B and/or T lymphopoiesis. Indeed, overexpression of miR-150 in hematopoietic stem cells blocked B lymphopoiesis by inhibiting the transition from the pro-B to the pre-B cell stage and greatly impaired the formation of mature B cells, but had little effect on the formation of either mature CD8- and CD4-positive T cells or granulocytes or macrophages (Zhou et al., 2007). This observation was consistent with another study, which also proved the importance of miR-150 in B cell formation using gain- and loss-of function mouse models (Xiao et al., 2007). Further, Xiao et al. (2007) demonstrated a transcript factor-Myb, which is highly expressed in lymphocyte progenitors, might be a crucial target of miR-150. Transgenic mice lacking c-Myb looked very similar to the transgenic mice with ectopic expression of miR-150 in the progenitors-abnormal B cell development and wholesale loss of B1 cells (Thomas et al., 2005). Although Myb has a key role in both B cell and T cell development, overexpression or deletion of miR-150 in mice affects only the development of B cells, but not T cells.

Several studies have proved B cells require miR-155 for normal production of isotype-switched, high-affinity antibodies and for a memory response. MiR-155 is processed from the third exon of *BIC* (B cell integration cluster) RNA. Transcripts of miR155 and BIC transcripts have been shown to accumulate in human B cell lymphoma, especially diffuse large B cell lymphomas (Eis et al., 2005), Hodgkin lymphomas (Kluiver et al., 2005), and subsets of Burkitt lymphomas (Kluiver et al., 2006). These reports provide indirect evidence that miR155 may play a role in B cell development and lymphomagenesis. The most direct evidence so far for the ability of miRNAs influencing immune responses was developed animal models with both ectopic expression and knockout of candidate miRNA. A study in Eμ-miR 155 transgenic mice revealed that the role of miR155 is to induce polyclonal expansion, and later develop a frank B cell malignancy (Costinean et al., 2006).

More recently, two groups proved the requirement of miR-155 for normal immune function using *bic*/miR-155 mutant/deficient mice. Rodriguez et al. (2007) found this knockout mice are immunodeficient and fail to develop a protective response to virulent *Salmonella typhimurium* infection after immunization with a non-virulent strain of this bacteria. As for B lymphocytes, immunized knockout mice produced significantly reduced amounts of IgM and switched antigen-specific antibodies, indicative of impaired B cell responses. Similarly, Thai et al. (2007) found in mice ectopically expressing miR-155, this miRNA control the germinal center B-cell response in part by regulating cytokine production of active B cells. In another study, B cells lacking miR-155 generated reduced extrafollicular and germinal center responses and failed to produce high-affinity IgG1 antibodies (Vigorito et al., 2007). Moreover, deregulation of PU.1 was found to be likely a contributing factor to the phenotype observed in miR-155-deficient mice (Vigorito et al., 2007).

Perspective

Although miRNAs were only discovered little more than a decade ago, the importance and broad roles of these noncoding small RNAs in a wide variety of biological processes, including embryogenesis, differentiation, proliferation and apoptosis, signal transduction as well as carcinogenesis, have become clear (Ke et al., 2003; Bartel, 2004; He and Hannon, 2004; Lu et al., 2005; Miska, 2005; Kapsimali et al., 2007). Recent years, investigators began to focus on the impact of these tiny players on complicated immune systems, and lots of miRNAs have been identified to involve in the regulation of immune responses, especially several miRNAs (miR155, miR181, miR150, and miR223, etc.) (Dahlberg and Lund, 2007; Li et al., 2007; Rodriguez et al., 2007; Xiao et al., 2007; Johnnidis et al., 2008) showed the central role in modulating immune system differentiation and immune responses. In addition, with the successful construction of miRNA-specific transgenic animals and knock-outs, immunologists can explore the roles and mechanisms of miRNA more conveniently. Present studies have showed the partial profiles of miRNAs in hematopoietic and immune cells, and relative signal pathways. However, the impact of miRNAs on the immune systems has yet to be fully understood and many questions still to be answered. Much more work need to be done to find the mechanism and function of miRNAs in the complex molecular network regulating the development and function of the immune system, which will provide not only deep understanding of immune homeostasis but also novel therapeutic approach in the treatment of inflammatory disease, autoimmune diseases, certain leukemias and other blood-related pathological disorders.

Acknowledgments

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