

## Invited Mini Review

## MiR-146 and miR-125 in the regulation of innate immunity and inflammation

Hye-Mi Lee<sup>1</sup>, Tae Sung Kim<sup>1,2</sup> & Eun-Kyeong Jo<sup>1,2,\*</sup><sup>1</sup>Department of Microbiology and <sup>2</sup>Department of Medical Science, Chungnam National University School of Medicine, Daejeon 35015, Korea

Innate immune responses are primary, relatively limited, and specific responses to numerous pathogens and toxic molecules. Protein expression involved in these innate responses must be tightly regulated at both transcriptional level and post-transcriptional level to avoid the development of excessive inflammation that can be potentially harmful to the host. MicroRNAs are small noncoding RNAs (~22 nucleotides [nts]) that participate in the regulation of numerous physiological responses by targeting specific messenger RNAs to suppress their translation. Recent work has shown that several negative regulators of transcription including microRNAs play important roles in inhibiting the exacerbation of inflammatory responses and in the maintenance of immunological homeostasis. This emerging research area will provide new insights on how microRNAs regulate innate immune signaling. It might show that dysregulation of microRNA synthesis is associated with the pathogenesis of inflammatory and infectious diseases. In this review, we focused on miR-146 and miR-125 and described the roles these miRNAs in modulating innate immune signaling. These microRNAs can control inflammatory responses and the outcomes of pathogenic infections. [BMB Reports 2016; 49(6): 311-318]

## INTRODUCTION

As an immediate defense mechanism in response to invading pathogens, almost all living organisms including mammals, plants, and insects possess innate immune responses. The innate immune system can recognize many pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) using various pattern-recognition receptors (PRRs). There are two major types of PRRs: membrane-bound

and cytosolic. They can recognize various PAMPs and DAMPs. Intracellular signaling triggered by PRR engagement plays a crucial role in the mounting of resistance to bacterial and viral infections via synthesizing immune mediators and antimicrobial chemicals.

MicroRNAs are small non-coding RNAs with 19-23 nucleotides in length. They can regulate mRNA expression by binding to the 3'-untranslated regions (3'-UTRs) of target mRNAs. Post-transcriptional regulatory functions of miRNAs include mRNA degradation, mRNA decay, or suppression of mRNA translation (1-4). MicroRNAs (miRNA) can influence the expression of many mRNAs, thus extensively modulating various physiological and pathological responses (3, 5). MiRNAs serve as important responders and contribute to the regulation of defense and inflammatory responses of the host (6-8).

Increasing evidence supports the idea that aberrant expression of miRNAs is associated with various aspects of local and systemic inflammatory and autoimmune diseases (9-11). Accumulating knowledge on how miRNAs regulate toll-like receptor (TLR) immune signaling will provide novel therapeutic strategies to control various inflammatory diseases (11-13). In this review, we will focus on the roles that miRNAs play and the molecular mechanisms involved in their regulation of excessive inflammatory responses that may potentially develop when the innate host defense system is deployed against pathogenic organisms and other dangerous stimuli.

## RESULTS

**General aspects of innate immunity: an overview of TLR signaling**

Many innate immune receptors recognize PAMPs and trigger intracellular signaling cascades essential for stimulating early (and ultimately successful) host defenses against infectious challenges (14). Of various innate immune receptors, the following two important membrane-bound receptors will be focused in this review: TLRs and nucleotide-binding oligomerization domain-like receptors (15, 16). It is becoming clear that many pattern-recognition receptors engage in crosstalk and orchestrate the protective immunity and inflammatory responses during infections (16). Signaling of the major

\*Corresponding author. Tel: +82-42-580-8243; Fax: +82-42-585-3686; E-mail: hayoungj@cnu.ac.kr

<http://dx.doi.org/10.5483/BMBRep.2016.49.6.056>

Received 16 March 2016

**Keywords:** Inflammation, Innate immune, MicroRNA, miR-125, miR-146

intracellular signaling pathways triggered by these PRRs will culminate in the activation of innate immune effectors and inflammatory mediators during infections (15, 17).

TLRs are the best characterized PRRs. They can recognize numerous ligands of a wide range of pathogens, including bacteria, viruses, fungi, and protozoa. They can also bind to toxic molecules derived from host tissues or cells. Each TLR has an extracellular ligand-binding domain, a transmembrane domain, and a cytosolic signaling domain termed toll-interleukin 1 receptor homology domain (TIR) (15). TLR signaling triggered by ligand engagement will cause homotypic binding of the TIR domain to partner domains within signaling adaptors. All TLRs except TLR3 interact with adaptor myeloid differentiation factor 88 (MyD88) that recruits members of the IL-1R-associated kinase (IRAK) family. IRAK proteins will then interact with E3 ubiquitin ligase and tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) to self-polyubiquitinate and recruit TAK1-binding proteins (TABs) 1-3. A TRAF6-mediated association of TAB2 and TAB3 with ubiquitin will trigger the activation of TAK1 (18). Such activation is required for the phosphorylation of inhibitor of  $\kappa$  light polypeptide gene enhancer in B-cells kinase (IKK) complex proteins (IKK- $\alpha$ , IKK- $\beta$ , and IKK- $\gamma$ ) and p38 kinases via activating mitogen-activated protein kinase kinase (MKK) 3, MKK6, and MKK7. IKK complex-mediated phosphorylation of I- $\kappa$ B will result in the ubiquitination and subsequent degradation of I- $\kappa$ B, which then triggers nuclear translocation of nuclear factor (NF)- $\kappa$ B. In cells of the innate immune system, NF- $\kappa$ B induces the expression of genes encoding many pro-inflammatory cytokines and costimulatory molecules (19). In addition, another adaptor molecule called TIR-domain-containing adapter-inducing interferon- $\beta$  protein can interact with both TLR3 and TLR4 and activate a TRAF3-dependent signaling cascade that in turn will trigger the activation of TRAF family member-associated NF- $\kappa$ B activator (TRAF)-binding kinase 1 (TBK1) and the inhibitor of NF- $\kappa$ B kinase (IKKi). TBK1/IKKi complex will phosphorylate and translocate transcription factor IRF3 into the nucleus, thus activating the transcription of interferon- $\beta$  gene (15).

TAK1 also activates several mitogen-activated protein kinases (MAPK) pathways, including ERK1/2, p38, and JNK MAPK pathways (15, 20, 21). MAPK activation requires signaling cascade containing at least three kinases. TAK1 serves as a MAPK kinase kinase (MAP3K) within p38 and JNK pathways crucial to the generation of immune mediators (15, 21, 22). In addition, TAK1 regulates the activation of COT/Tpl2 (the only MAP3K that can activate MAPK kinases 1 and 2), leading to the activation of ERK1/2 pathway. This ERK1/2 pathway plays roles in both innate immune response and inflammatory response (23). All three MAPKs will phosphorylate downstream targets (including other kinases and transcription factors) which in turn regulate the transcription of many genes including those encoding proinflammatory cytokines (15, 21, 22). In this review, we will

describe the recent advances in our understanding on how miRNAs regulate innate immune responses triggered by TLRs, which in turn activate host immune defenses and inflammation. The roles played by miRNAs, the mechanisms by which miRNAs regulate the components of TLR-signaling pathways, and the relevance of such actions in the context of host defense and inflammatory disease will be discussed.

### Overview of miRNA biogenesis

Biogenesis, roles in target recognition and post-transcriptional gene regulation, and other biological functions of miRNAs have been discussed extensively in many earlier reviews (24-26). Thus, we will only briefly summarize miRNA biogenesis here. MicroRNAs are well-known small noncoding RNA molecules that control gene expression by interacting with mRNAs. The first miRNA was discovered in *Caenorhabditis elegans* in 1993 (27, 28). In mammalian cells, miRNAs are predicted to target more than 30% of protein-encoding mRNAs. Therefore, miRNAs regulate genes in almost all biological processes. By base-pairing to mRNAs, miRNAs can inhibit the translation or increase mRNA turnover (25). To date, many miRNAs of mammalian cells have been identified and studied. miRNAs can modulate a variety of essential biological responses to physiological, stressful, and pathogenic conditions (26, 29). miRNAs are crucial regulators of a variety of cellular processes. It is now becoming clear that aberrant miRNA expression is a general signature of many human diseases, including cancer, metabolic disorder, and immune disease (30-32).

During miRNA biogenesis, mature miRNAs are generated from long primary miRNA transcripts (pri-miRNAs) via a sequence of biochemical steps (24, 33). In the nucleus, processing of a pri-miRNA into a pre-miRNA (~70 nts) involves recognition of the stem-loop structure of miRNA by an RNase III/Drosha-DiGeorge syndrome critical region gene 8 complex (34, 35). After export of pre-miRNA into the cytoplasm by exportin 5 (36, 37), Dicer (a member of the RNase III superfamily of ribonucleases) will interact with RNA-induced silencing complex (RISC) loading complex and process the pre-miRNA to release a ~22-nt miRNA-miRNA\* duplex with a 2-nt 3' overhang at either end (34, 38, 39). The double strands of this duplex are separated by an RNA helicase (40). One miRNA strand (the guide strand) of the duplex is then loaded into an Argonaute-containing RISC (41, 42). Finally, the single-stranded mature miRNA will pair with mRNAs by interacting with the 3' UTRs of those mRNAs (43). This will cause translational repression and/or mRNA destabilization and degradation (4, 5, 24).

### Functional importance of miRNAs in innate immunity

It is becoming clear that miRNAs play major roles in the regulation of immune cell differentiation, release of inflammatory mediators, host defense, and various immunological diseases (9, 44, 45). Many miRNAs that are rapidly induced by

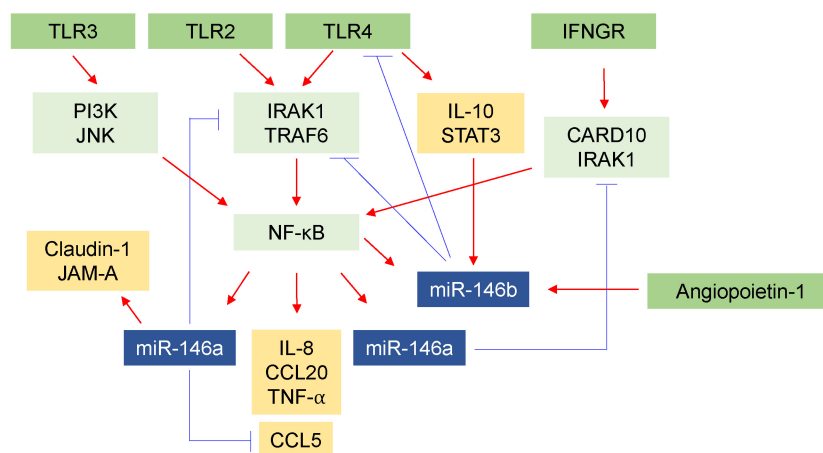
the activation of innate immune system have been discussed extensively in several excellent reviews (11, 13). Therefore, we will not visit these topics here. In this review, we will briefly focus on miR-146a, miR-146b, miR-125a, miR-125b, and their regulation of innate immune response, inflammatory response, and antimicrobial response (46-52).

**MiR-146 and innate immune response regulation:** MiR-146a, a NF- $\kappa$ B-associated gene (46), has been studied extensively for its role in innate immunity (53). This miRNA plays an essential role in negative regulation of the production of proinflammatory cytokines, thus modulating the severity of inflammatory response (54). Earlier studies have suggested that miR-146a is involved in linking innate immune response to oncogenic transformation (55-57). MiR-146a plays a critical role in regulating the proliferation of immune cells and inhibiting inflammatory responses (56, 57). An miR-146a deficiency in mice is associated with chronic dysregulation of NF- $\kappa$ B signaling, yielding a phenotype with characteristics of myeloid malignancy (57) (Fig. 1). Indeed, the gene encoding miR-146a (encoded on chromosome 5q33.3) has been reported to be absent in hematopoietic progenitor cells (deletion mutations in a segment of chromosome 5q) of many myelodysplastic syndrome (MDS) patients with 5q-syndrome (58).

Both miR-146a and miR-146b can regulate inflammatory responses by targeting mRNAs encoding IRAK-1 and TRAF6 (46, 53, 59, 60). An *in vivo* deficiency in miR-146a can trigger macrophage hyperactivation, elevate the systemic response to endotoxin (lipopolysaccharide, LPS), and predispose to the development of an autoimmune phenotype later in life (56). In addition, one study has shown that miR-146a is characteristically upregulated in LPS-adapted human monocytic cells,

suggesting that miR-146a might be a key regulator in endotoxin tolerance (61).

Recent *in vivo* studies using a lentivirus expressing miR-146a (LmiR-146a) have shown that miR-146a is essential to the prevention of sepsis-induced NF- $\kappa$ B signaling and the generation of inflammatory cytokines as well as the inhibition of IRAK and TRAF6 expression in the myocardium, thus attenuating cardiac dysfunction often associated with sepsis (62). TLR3-stimulation of human nasal epithelial cells can induce miR-146a synthesis via phosphoinositide 3-kinase (PI3K), JNK, and NF- $\kappa$ B pathways (63). Notably, miR-146a plays an important role in the expression of tight junction proteins claudin-1 and JAM-A, suggesting that miR-146a is essential to the maintenance of tight junction barrier and innate immune defense (63). In primary human keratinocytes, miR-146a can inhibit the development of NF- $\kappa$ B-dependent inflammatory responses by directly targeting recruitment (by the upstream nuclear factor kappa B) of the following three signal transducers: caspase domain-containing protein 10, IL-1 receptor-associated kinase 1, and chemokine (C-C motif) ligand (CCL) 5 (64). Moreover, TLR2 stimulation can trigger sustained expression of miR-146a, which in turn will suppress the synthesis of IL-8, CCL20, and TNF- $\alpha$  in primary human keratinocytes (65). In addition, activation of TLR4 signaling can upregulate miR-146b expression in human monocytes via the action of IL-10-mediated STAT3-dependent pathway (51). In turn, miR-146b can negatively regulate LPS-mediated production of many proinflammatory cytokines and chemokines. MiR-146b fulfills these roles by targeting many components of signaling pathways, including TLR4, MyD88, IRAK-1, and TRAF6 (51). In human umbilical vein endothelial



**Fig. 1.** The levels of miR-146a and miR-146b are regulated by innate immune signaling. The levels of miR-146a and miR-146b are up- or down- regulated by TLR-induced intracellular signaling. TLR3 activates PI3K-JNK and NF- $\kappa$ B signal pathways, which up-regulates the level of miR-146a. TLR2 signaling activates the IRAK1-TRAF6-NF $\kappa$ B pathway that enhances inflammatory responses through producing proinflammatory cytokines and chemokines. The activation of miR-146a decreases proinflammatory cytokines via inhibiting IRAK1 and TRAF6. MiR-146b level is up-regulated by TLR4 signaling through STAT3 and IL-10. Angiopoietin-1 induces miR-146b expression. The expression of miR-146a is negatively regulated by IFN-induced CARD10-IRAK1 pathway.

cells (HUVECs), prolonged expression of angiopoietin-1 can significantly decrease LPS-induced IRAK1 and TRAF6 levels via upregulating miR-146b-5p expression. Interestingly, angiopoietin-1 cannot influence the expression levels of miR-146a or miR-146b-3 in HUVECs (66). Together, these studies have demonstrated that both miR-146a and miR-146b are critical negative regulators of the activities of various cell types in the innate immune system, thus preventing the development of harmful inflammatory responses and promoting the maintenance of homeostatic conditions (Fig. 1).

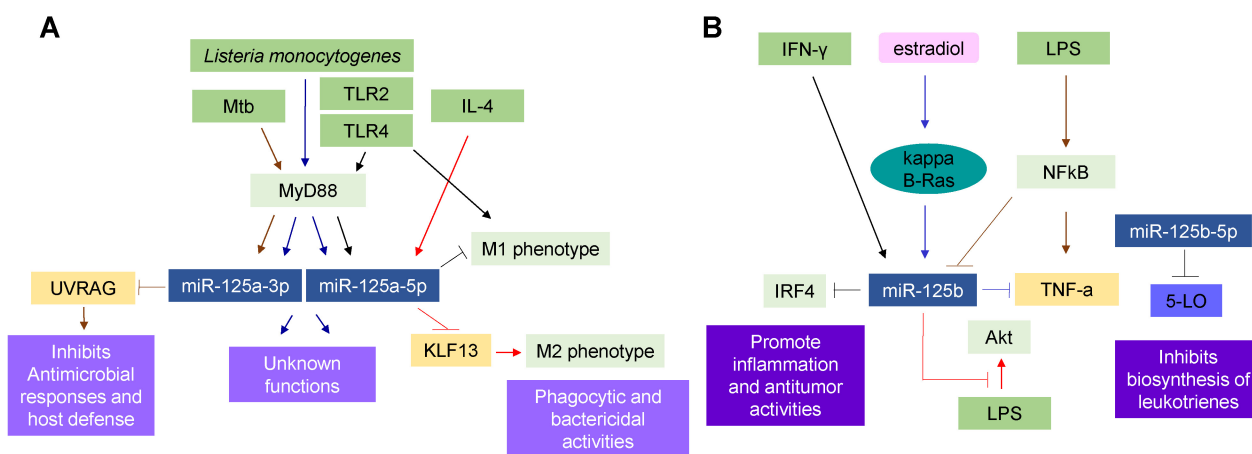
**MiR-125 and innate immune regulation:** Recent studies have shown that miR-125a-5p plays an important role in inhibiting the classical M1-type activation induced by LPS stimulation. In addition, miR-125a-5p can promote IL-4-induced expression of the alternative M2 phenotype by targeting KLF13, a transcriptional factor that is active during T lymphocyte activation and inflammation (50). Additionally, miR-125a-5p can suppress the phagocytic and bactericidal activities associated with macrophage M1 functionality (50). Earlier studies have shown that the expression levels of both miR-125a-3p/5p and miR-146a are regulated at transcriptional level after *Listeria monocytogenes* infection that altered the miRNA profiles of host macrophages (67). The precise functions of these miRNAs remain unclear. Our recent study has shown that miR-125a-3p can inhibit antimicrobial responses and host defenses against mycobacterial infection by targeting the gene encoding autophagy UV radiation-resistance-associated protein (68). Together, these data suggest that miR-125a may inhibit innate

macrophage responses by regulating macrophage differentiation, inflammation, and autophagy (Fig. 2A).

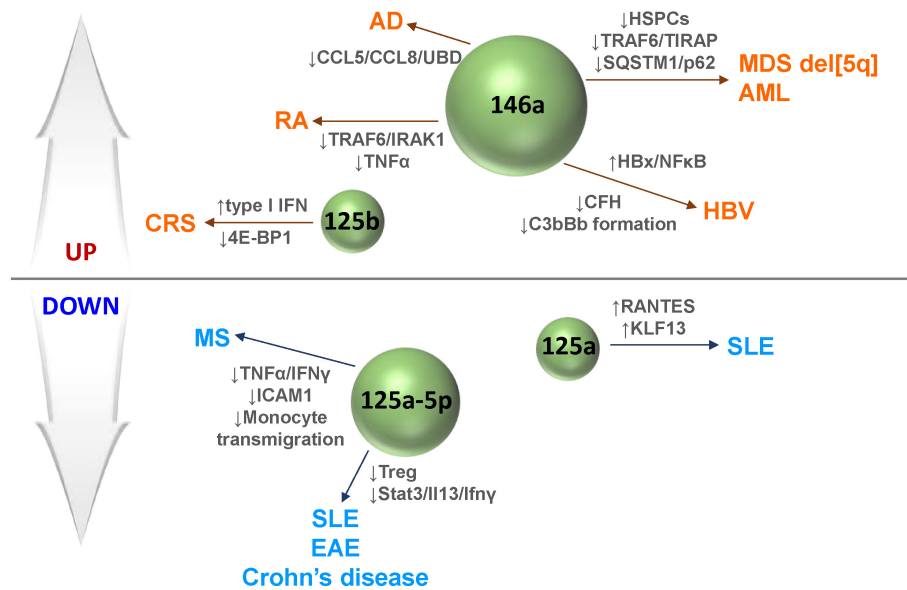
Interestingly, the expression of miR-125b-5p (which has the same core sequence as miR-125a-5p) is modulated by NF- $\kappa$ B signaling. miR-125b-5p targets the 3'UTR region of TNF- $\alpha$  gene to negatively regulate the inflammatory response (49) (Fig. 2B). The expression levels of both miR-125b and miR-155 are negatively regulated by LPS-induced Akt1 activation. This can regulate the extent of endotoxin tolerance/sensitivity in mice (69). In addition, LPS stimulation of human macrophages can suppress the expression of miR-125b. However, estradiol pretreatment can eliminate this effect, thus enhancing the stability of  $\kappa$ B-Ras2-encoding mRNA.  $\kappa$ B-Ras2 is a key inhibitor of NF- $\kappa$ B signaling (70). Recent data have shown that miR-125b-5p can directly target and inhibit the expression of a gene encoding 5-lipoxygenase, a key enzyme in the biosynthesis of leukotrienes essential for both innate immune responses and inflammatory processes (71). However, another study has reported that miR-125b can promote macrophage-mediated inflammation, increase the expression of co-stimulatory factor, and enhance antitumor activities by targeting Interferon regulatory factor 4 (IRF4) (72) (Fig. 2B). These data suggest that miRNAs play different roles in diverse biological contexts.

### Clinical implications of miR-146 and miR125 expression in patients with inflammatory diseases

Dysregulation of miRNA expression is associated with the pathogenesis of many human diseases, indicating that miRNAs



**Fig. 2.** The roles of miR-125a and miR-125b in the regulation of innate and inflammatory responses. (A) MiR-125a-3p and miR-125a-5p negatively regulate host defense and bactericidal activity. *Mycobacterium tuberculosis* (Mtb), *Listeria monocytogenes*, and several TLR ligands activate innate immune pathways involving MyD88. *Listeria monocytogenes* activates the expression of miR-125a-3p and miR-125a-5p. Mtb infection upregulates miR-125a-3p levels and inhibits UVRAG expression, which modulates antimicrobial responses and host defense. MiR-125a-5p inhibits M1 phenotype activated by TLR ligands such as Pam3CSK4 (Pam3) and LPS. IL-4 induces the expression of miR-125a-5p and promotes M2 phenotype. MiR-125a-5p may control phagocytic and bactericidal activities through inhibiting KLF3. (B) LPS-induced Akt signal pathway is down-regulated by miR-125b. 5-Lipoxygenase (5-LO) expression is decreased by miR-125b-5p. MiR-125b positively regulates inflammatory responses. IFN-gamma stimulates the activation of miR-125b following inhibition of IRF4, which promotes inflammation and antitumor activities.



**Fig. 3.** Dysregulated levels of miR-125 and miR-146 in various infectious and inflammatory diseases. MiR-125 and miR-146 levels are upregulated or downregulated in various infectious and inflammatory diseases. Upper panel: up-regulated miRNA mediates chronic rhinosinusitis (CRS), Rheumatoid arthritis (RA), atopic dermatitis (AD), Myelodysplastic syndromes (MDS), acute myeloid leukemia (AML), and Hepatitis B virus infection (HBV). Lower panel: down-regulated miRNA mediates multiple sclerosis (MS), systemic lupus erythematosus (SLE), experimental autoimmune encephalomyelitis (EAE), systemic lupus erythematosus (SLE), and Crohn's disease.

may serve as novel diagnostic or therapeutic targets. The relevance of miR-146a in disease contexts is now well-accepted. Early studies have shown that miR-146a expression levels are significantly increased in peripheral blood mononuclear cells from rheumatoid arthritis patients, although the levels of TRAF6 and IRAK1 (two targets of miR-146a) in patients are similar to those in healthy controls (73). MiR-146a expression levels are elevated in keratinocytes and skin disease lesions of patients with atopic dermatitis. In human primary keratinocytes, miR-146a can inhibit the expression of many proinflammatory cytokines and chemokines, including CCL5, CCL8, and ubiquitin D. In addition, it has been reported that miR-146a-deficient mice exhibit exacerbated inflammation with accumulation of infiltrating cells in the dermis and increased skin inflammation (64). Moreover, aberrant expression of miR-146a (the gene of which resides on chromosome 5q) is associated with dysregulation of innate immune response and the development of clinical 5q-syndrome, a subtype of myelodysplastic syndrome (58). Triple deletion of genes encoding TRAF-interacting protein, forkhead-associated domain B protein (TIFAB), and miR-146a has been shown to increase the level of TRAF6 protein, sustain TRAF6-mediated signaling, and trigger hematopoietic dysfunction, partly explaining the pathogenesis of high-risk MDS and acute myeloid leukemia associated with chromosome 5q deletions (74, 75). Recent studies have revealed an interrelationship between miR-146a synthesis and the pathogenesis of hepatic

injuries developed after hepatitis B virus (HBV) infection (76). MiR-146a expression levels are upregulated in HBV-infected cells, infected mice per se, and human HBV-infected patients. HBV X protein-induced NF- $\kappa$ B signaling is required for the expression of miR-146a, which in turn downregulates the level of the mRNA encoding complement factor H, a negative regulator of the alternative pathway of complement activation. Thus, miR-146a may play a role in the immunopathogenesis of chronic hepatitis B infection (76).

Compared to miR-146a, much less is known about the roles of miR-125a-5p in inflammatory disease. MiR-125a levels are significantly reduced in patients with systemic lupus erythematosus (SLE). It has been reported that MiR-125a overexpression can inhibit the levels of RANTES (regulated on activation, normal T-cell expressed and secreted) inflammatory chemokines by controlling the expression levels of a predicted target gene *KLF13* (77). In addition, miR-125a-5p expression is reduced in active lesions of multiple sclerosis patients (78). Further analysis has shown that miR-125a-5p is required to enhance the tightness of the brain endothelial barrier and to stimulate the formation of thick cell-cell junctional complexes in the brain endothelium (78). Recently, it has been shown that miR-125a is essential in terms of regulatory T cell function because this miRNA can suppress the expression of effector T cell factors. MiR-125a levels are downregulated in peripheral CD4<sup>+</sup> T cells of patients with autoimmune SLE and Crohn's disease (79). MiR-125b has clinical relevance in patients with

chronic eosinophilic rhinosinusitis and nasal polyps because the levels of miR-125b are elevated in patients and such elevation can regulate the extent of inflammation in sinonasal mucosal samples of these patients (80). MiR-125b upregulation can elevate IFN- $\beta$  mRNA levels in airway epithelial cells by targeting EIF4E-binding protein 1 (4E-BP1), thus exacerbating mucosal eosinophilia (80). Thus, miRNAs can modulate the expression levels of genes involved in almost all cellular functions (Fig. 3). Further exploration of the roles they play in pathogenesis and defenses of innate immune responses against infections will yield miRNA-derived therapeutics useful for treating many human inflammatory disorders.

## DISCUSSION

Our understanding on the roles of miRNAs in innate immune signaling and inflammation has advanced rapidly and extensively over the past several years. Based on data from recent studies, it can be concluded that both miR-144 and miR-125 are important regulators in innate immune responses. They play many roles in the regulation of innate immune responses to both pathogenic infections and nonpathogenic inflammation. Identification of other miRNAs that may modulate the (complex) activation of innate immune responses remains a topic of interest. MiRNAs are emerging as potential diagnostic markers. They are important translational regulators in many human disease states, including inflammation and infectious diseases. Unraveling the miRNA network is important so that we can understand the pathogenesis of many human inflammatory diseases to develop potentially useful treatments. Accumulating basic research data on miR-146 and miR-125 have identified their novel roles and the molecular mechanisms involved. Such work will lead to the development of new diagnostic and therapeutic strategies for immune disease and inflammation.

## ACKNOWLEDGEMENTS

We thank our current and past members of our laboratory for discussions and investigations that contributed to this article. This work was supported by a grant (NRF-2013R1A1A 2058116) of the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Republic of Korea. This work was also supported by a research fund (2015) of Chungnam National University. We apologize to colleagues whose work and publications could not be referenced owing to space constraints. The authors declare no financial conflict of interests.

## REFERENCES

1. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116, 281-297

2. Fabian MR, Sonenberg N and Filipowicz W (2010) Regulation of mRNA translation and stability by microRNAs. *Annu Rev Biochem* 79, 351-379
3. Friedman JM and Jones PA (2009) MicroRNAs: critical mediators of differentiation, development and disease. *Swiss Med Wkly* 139, 466-472
4. Guo H, Ingolia NT, Weissman JS and Bartel DP (2010) Mammalian microRNAs predominantly act to decrease target mRNA levels. *Nature* 466, 835-840
5. Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. *Cell* 136, 215-233
6. O'Connell RM, Rao DS, Chaudhuri AA and Baltimore D (2010) Physiological and pathological roles for microRNAs in the immune system. *Nat Rev Immunol* 10, 111-122
7. El Gazzar M and McCall CE (2012) MicroRNAs regulatory networks in myeloid lineage development and differentiation: regulators of the regulators. *Immunol Cell Biol* 90, 587-593
8. Iliopoulos D (2014) MicroRNA circuits regulate the cancer-inflammation link. *Sci Signal* 7, pe8
9. Baltimore D, Boldin MP, O'Connell RM, Rao DS and Taganov KD (2008) MicroRNAs: new regulators of immune cell development and function. *Nat Immunol* 9, 839-845
10. Pauley KM, Cha S and Chan EK (2009) MicroRNA in autoimmunity and autoimmune diseases. *J Autoimmun* 32, 189-194
11. Dai R and Ahmed SA (2011) MicroRNA, a new paradigm for understanding immunoregulation, inflammation, and autoimmune diseases. *Transl Res* 157, 163-179
12. Olivieri F, Procopio AD and Montgomery RR (2014) Effect of aging on microRNAs and regulation of pathogen recognition receptors. *Curr Opin Immunol* 29, 29-37
13. O'Neill LA, Sheedy FJ and McCoy CE (2011) MicroRNAs: the fine-tuners of Toll-like receptor signalling. *Nat Rev Immunol* 11, 163-175
14. Kumar H, Kawai T and Akira S (2011) Pathogen recognition by the innate immune system. *Int Rev Immunol* 30, 16-34
15. Kawai T and Akira S (2010) The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol* 11, 373-384
16. Kawai T and Akira S (2011) Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity* 34, 637-650
17. Ishii KJ, Koyama S, Nakagawa A, Coban C and Akira S (2008) Host innate immune receptors and beyond: making sense of microbial infections. *Cell Host Microbe* 3, 352-363
18. Clark K, Nanda S and Cohen P (2013) Molecular control of the NEMO family of ubiquitin-binding proteins. *Nat Rev Mol Cell Biol* 14, 673-685
19. Kawai T and Akira S (2007) Signaling to NF-kappaB by Toll-like receptors. *Trends Mol Med* 13, 460-469
20. Arthur JS and Ley SC (2013) Mitogen-activated protein kinases in innate immunity. *Nat Rev Immunol* 13, 679-692
21. Coll RC and O'Neill LA (2010) New insights into the regulation of signalling by toll-like receptors and nod-like

- receptors. *J Innate Immun* 2, 406-421
22. Symons A, Beinke S and Ley SC (2006) MAP kinase kinase kinases and innate immunity. *Trends Immunol* 27, 40-48
  23. Cohen P (2014) The TLR and IL-1 signalling network at a glance. *J Cell Sci* 127, 2383-2390
  24. Kim VN, Han J and Siomi MC (2009) Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol* 10, 126-139
  25. Filipowicz W, Bhattacharyya SN and Sonenberg N (2008) Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet* 9, 102-114
  26. Mendell JT and Olson EN (2012) MicroRNAs in stress signaling and human disease. *Cell* 148, 1172-1187
  27. Lee RC, Feinbaum RL and Ambros V (1993) The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 75, 843-854
  28. Wightman B, Ha I and Ruvkun G (1993) Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in *C. elegans*. *Cell* 75, 855-862
  29. Olive V, Minella AC and He L (2015) Outside the coding genome, mammalian microRNAs confer structural and functional complexity. *Sci Signal* 8, re2
  30. Lu J, Getz G, Miska EA et al (2005) MicroRNA expression profiles classify human cancers. *Nature* 435, 834-838
  31. Negrini M, Ferracin M, Sabbioni S and Croce CM (2007) MicroRNAs in human cancer: from research to therapy. *J Cell Sci* 120, 1833-1840
  32. McGregor RA and Choi MS (2011) microRNAs in the regulation of adipogenesis and obesity. *Curr Mol Med* 11, 304-316
  33. Lee YS and Dutta A (2009) MicroRNAs in cancer. *Annu Rev Pathol* 4, 199-227
  34. Lee Y, Ahn C, Han J et al (2003) The nuclear RNase III Drosha initiates microRNA processing. *Nature* 425, 415-419
  35. Han J, Lee Y, Yeom KH, Kim YK, Jin H and Kim VN (2004) The Drosha-DGCR8 complex in primary microRNA processing. *Genes Dev* 18, 3016-3027
  36. Lund E, Güttinger S, Calado A, Dahlberg JE and Kutay U (2004) Nuclear export of microRNA precursors. *Science* 303, 95-98
  37. Yi R, Qin Y, Macara IG and Cullen BR (2003) Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev* 17, 3011-3016
  38. Bernstein E, Caudy AA, Hammond SM and Hannon GJ (2001) Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* 409, 363-366
  39. Hutvagner G, McLachlan J, Pasquinelli AE, Bálint E, Tuschl T and Zamore PD (2001) A cellular function for the RNA-interference enzyme Dicer in the maturation of the *let-7* small temporal RNA. *Science* 293, 834-838
  40. Salzman DW, Shubert-Coleman J and Furneaux H (2007) P68 RNA helicase unwinds the human *let-7* microRNA precursor duplex and is required for *let-7*-directed silencing of gene expression. *J Biol Chem* 282, 32773-32779
  41. Chendrimada TP, Gregory RI and Kumaraswamy E et al (2005) TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature* 436, 740-744
  42. Kawamata T and Tomari Y (2010) Making RISC. *Trends Biochem Sci* 35, 368-376
  43. Witkos TM, Koscianska E and Krzyzosiak WJ (2011) Practical Aspects of microRNA Target Prediction. *Curr Mol Med* 11, 93-109
  44. Sonkoly E, Stähle M and Pivarcsi A (2008) MicroRNAs and immunity: novel players in the regulation of normal immune function and inflammation. *Semin Cancer Biol* 18, 131-140
  45. Lindsay MA (2008) microRNAs and the immune response. *Trends Immunol* 29, 343-351
  46. Taganov KD, Boldin MP, Chang KJ and Baltimore D (2006) NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci U S A* 103, 12481-12486
  47. O'Connell RM, Taganov KD, Boldin MP, Cheng G and Baltimore D (2007) MicroRNA-155 is induced during the macrophage inflammatory response. *Proc Natl Acad Sci U S A* 104, 1604-1609
  48. Moschos SA, Williams AE, Perry MM, Birrell MA, Belvisi MG and Lindsay MA (2007) Expression profiling in vivo demonstrates rapid changes in lung microRNA levels following lipopolysaccharide-induced inflammation but not in the anti-inflammatory action of glucocorticoids. *BMC Genomics* 8, 240
  49. Tili E, Michaille JJ, Cimino A et al (2007) Modulation of miR-155 and miR-125b levels following lipopolysaccharide/TNF-alpha stimulation and their possible roles in regulating the response to endotoxin shock. *J Immunol* 179, 5082-5089
  50. Banerjee S, Cui H, Xie N et al (2013) miR-125a-5p regulates differential activation of macrophages and inflammation. *J Biol Chem* 288, 35428-35436
  51. Curtale G, Mirolo M, Renzi TA, Rossato M, Bazzoni F and Locati M (2013) Negative regulation of Toll-like receptor 4 signaling by IL-10-dependent microRNA-146b. *Proc Natl Acad Sci U S A* 110, 11499-11504
  52. Li T, Morgan MJ, Choksi S, Zhang Y, Kim YS and Liu ZG (2010) MicroRNAs modulate the noncanonical transcription factor NF-kappaB pathway by regulating expression of the kinase IKKalpha during macrophage differentiation. *Nat Immunol* 11, 799-805
  53. Saba R, Sorensen DL and Booth SA (2014) MicroRNA-146a: A Dominant, Negative Regulator of the Innate Immune Response. *Front Immunol* 5, 578
  54. Perry MM, Moschos SA, Williams AE, Shepherd NJ, Larner-Svensson HM and Lindsay MA (2008) Rapid changes in microRNA-146a expression negatively regulate the IL-1beta-induced inflammatory response in human lung alveolar epithelial cells. *J Immunol* 180, 5689-5698
  55. Williams AE, Perry MM, Moschos SA, Larner-Svensson HM and Lindsay MA (2008) Role of miRNA-146a in the regulation of the innate immune response and cancer. *Biochem Soc Trans* 36, 1211-1215
  56. Boldin MP, Taganov KD, Rao DS et al (2011) miR-146a is a significant brake on autoimmunity, myeloproliferation, and cancer in mice. *J Exp Med* 208, 1189-1201

57. Zhao JL, Rao DS, Boldin MP, Taganov KD, O'Connell RM and Baltimore D (2011) NF-kappaB dysregulation in microRNA-146a-deficient mice drives the development of myeloid malignancies. *Proc Natl Acad Sci U S A* 108, 9184-9189
58. Starczynowski DT, Kuchenbauer F, Argiropoulos B et al (2010) Identification of miR-145 and miR-146a as mediators of the 5q- syndrome phenotype. *Nat Med* 16, 49-58
59. Cui JG, Li YY, Zhao Y, Bhattacharjee S and Lukiw WJ (2010) Differential regulation of interleukin-1 receptor-associated kinase-1 (IRAK-1) and IRAK-2 by microRNA-146a and NF-kappaB in stressed human astroglial cells and in Alzheimer disease. *J Biol Chem* 285, 38951-38960
60. Park H, Huang X, Lu C, Cairo MS and Zhou X (2015) MicroRNA-146a and microRNA-146b regulate human dendritic cell apoptosis and cytokine production by targeting TRAF6 and IRAK1 proteins. *J Biol Chem* 290, 2831-2841
61. Nahid MA, Pauley KM, Satoh M and Chan EK (2009) miR-146a is critical for endotoxin-induced tolerance: IMPLICATION IN INNATE IMMUNITY. *J Biol Chem* 284, 34590-34599
62. Gao M, Wang X, Zhang X et al (2015) Attenuation of Cardiac Dysfunction in Polymicrobial Sepsis by MicroRNA-146a Is Mediated via Targeting of IRAK1 and TRAF6 Expression. *J Immunol* 195, 672-682
63. Miyata R, Kakuki T, Nomura K et al (2015) Poly(I:C) induced microRNA-146a regulates epithelial barrier and secretion of proinflammatory cytokines in human nasal epithelial cells. *Eur J Pharmacol* 761, 375-382
64. Rebane A, Runnel T, Aab A et al (2014) MicroRNA-146a alleviates chronic skin inflammation in atopic dermatitis through suppression of innate immune responses in keratinocytes. *J Allergy Clin Immunol* 134, 836-847 e811
65. Meisgen F, Xu Landén N and Wang A et al (2014) MiR-146a negatively regulates TLR2-induced inflammatory responses in keratinocytes. *J Invest Dermatol* 134, 1931-1940
66. Echavarría R, Mayaki D, Neel JC, Harel S, Sanchez V and Hussain SN (2015) Angiopoietin-1 inhibits toll-like receptor 4 signalling in cultured endothelial cells: role of miR-146b-5p. *Cardiovasc Res* 106, 465-477
67. Schnitger AK, Machova A, Mueller RU et al (2011) *Listeria monocytogenes* infection in macrophages induces vacuolar-dependent host miRNA response. *PLoS One* 6, e27435
68. Kim JK, Yuk JM, Kim SY et al (2015) MicroRNA-125a Inhibits Autophagy Activation and Antimicrobial Responses during Mycobacterial Infection. *J Immunol* 194, 5355-5365
69. Androulidaki A, Iliopoulos D, Arranz A et al (2009) The kinase Akt1 controls macrophage response to lipopolysaccharide by regulating microRNAs. *Immunity* 31, 220-231
70. Murphy AJ, Guyre PM and Pioli PA (2010) Estradiol suppresses NF-kappa B activation through coordinated regulation of let-7a and miR-125b in primary human macrophages. *J Immunol* 184, 5029-5037
71. Busch S, Auth E, Scholl F et al (2015) 5-lipoxygenase is a direct target of miR-19a-3p and miR-125b-5p. *J Immunol* 194, 1646-1653
72. Chaudhuri AA, So AY, Sinha N et al (2011) MicroRNA-125b potentiates macrophage activation. *J Immunol* 187, 5062-5068
73. Pauley KM, Satoh M, Chan AL, Bubb MR, Reeves WH and Chan EK (2008) Upregulated miR-146a expression in peripheral blood mononuclear cells from rheumatoid arthritis patients. *Arthritis Res Ther* 10, R101
74. Fang J, Barker B, Bolanos L et al (2014) Myeloid malignancies with chromosome 5q deletions acquire a dependency on an intrachromosomal NF-κB gene network. *Cell Rep* 8, 1328-1338
75. Varney ME, Niederkorn M, Konno H et al (2015) Loss of Tifab, a del(5q) MDS gene, alters hematopoiesis through derepression of Toll-like receptor-TRAF6 signaling. *J Exp Med* 212, 1967-1985
76. Li JF, Dai XP, Zhang W et al (2015) Upregulation of microRNA-146a by hepatitis B virus X protein contributes to hepatitis development by downregulating complement factor H. *MBio* 6, pii: e02459-02414
77. Zhao X, Tang Y, Qu B et al (2010) MicroRNA-125a contributes to elevated inflammatory chemokine RANTES levels via targeting KLF13 in systemic lupus erythematosus. *Arthritis Rheum* 62, 3425-3435
78. Reijerkerk A, Lopez-Ramirez MA, van Het Hof B et al (2013) MicroRNAs regulate human brain endothelial cell-barrier function in inflammation: implications for multiple sclerosis. *J Neurosci* 33, 6857-6863
79. Pan W, Zhu S, Dai D et al (2015) MiR-125a targets effector programs to stabilize Treg-mediated immune homeostasis. *Nat Commun* 6, 7096
80. Zhang XH, Zhang YN, Li HB et al (2012) Overexpression of miR-125b, a novel regulator of innate immunity, in eosinophilic chronic rhinosinusitis with nasal polyps. *Am J Respir Crit Care Med* 185, 140-151