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ABSTRACT

The innate immune responses are the primary, relatively limited, specific responses to numerous pathogens and toxic molecules. The expression of proteins involved in these innate responses must be tightly regulated at both the transcriptional and post-transcriptional levels to avoid the development of excessive inflammation, which is 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 afford new insights on how microRNAs regulate innate immune signaling and may show that dysregulation of microRNA synthesis is associated with the pathogenesis of inflammatory and infectious diseases. In this Review, we focus on miR-146 and miR-125; we describe the roles these miRNAs play in the modulation of innate immune signaling. These microRNAs control inflammatory responses and the outcomes of pathogenic infections.

INTRODUCTION

The human body deploys an immediate defense mechanism in response to invading pathogens; this is termed the innate immune response and is present in almost all living organisms including mammals, plants, and insects. The systems recognize many pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) using various pattern-recognition receptors (PRRs). Two major types of PRRs are known, the membrane-bound and cytosolic types; these recognize the various PAMPs and DAMPs. Intracellular signaling triggered by PRR engagement plays a crucial role in the mounting of resistance to bacterial and viral infections, achieved via synthesis of immune mediators and antimicrobial chemicals.

MicroRNAs are small non-coding RNAs 19-23 nucleotides in length that regulate mRNA expression by binding to the 3′-untranslated regions (3′-UTRs) of target gene mRNAs. The post-transcriptional regulatory functions of miRNAs feature mRNA degradation, mRNA decay, or suppression of mRNA translation (1-4). MicroRNAs influence the expression of many mRNAs and thus extensively modulate various physiological and pathological responses (3, 5). MiRNAs serve as important responders, contributing to regulation of the 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 allow novel therapeutic strategies for various inflammatory diseases to be developed (11-13). In this review, we focus on the roles played by miRNAs, and the molecular mechanisms involved, in the 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 stimulation of early (and ultimately successful) host defenses against infectious challenges (14). Of the various innate immune receptors, we will focus on two important membrane-bound receptors; these are the TLRs and nucleotide-binding oligomerization domain-like receptors (15, 16). It is becoming clear that many pattern-recognition receptors engage in crosstalk, orchestrating the protective immunity and inflammatory responses developing during infection (16). Signaling by the major intracellular signaling pathways triggered by these PRRs culminate in activation of innate immune effectors and inflammatory mediators during infection (15, 17).

TLRs, the best characterized PRRs, recognize numerous ligands on a wide range of pathogens, including bacteria, viruses, fungi, and protozoa; they 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 the Toll-interleukin 1 receptor homology domain (TIR) (15). TLR signaling triggered by ligand engagement causes homotypic binding of the TIR domain to partner domains within signaling adaptors. All TLRs, except for TLR3, act in combination with the adaptor myeloid differentiation factor 88 (MyD88), which recruits members of the IL-1R-associated kinase (IRAK) family. The IRAK proteins then interact with the E3 ubiquitin ligase tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6), which self-polyubiquitinates and recruits TAK1-binding proteins (TABs) 1–3. A TRAF6-mediated association of TAB2 and TAB3 with ubiquitin triggers TAK1 activation (18). Such activation is required for phosphorylation of inhibitor of κ light polypeptide gene enhancer in B-cells kinase (IKK) complex proteins (IKK-α, IKK-β, and IKK-γ) and p38 kinases via activation of mitogen-activated protein kinase kinase (MKK) 3, MKK6, and MKK7. IKK complex-mediated phosphorylation of I-κB results in ubiquitination and subsequent degradation of I-κB, triggering nuclear translocation of nuclear factor (NF)-

κB. In cells of the innate immune system, NF-κB induces the expression of genes encoding many pro-inflammatory cytokines and costimulatory molecules (19). In addition, another adaptor molecule, the TIR-domain-containing adapter-inducing interferon-β protein, which interacts with both TLR3 and TLR4, activates a TRAF3-dependent signaling cascade that in turn triggers activation of TRAF family member-associated NF-κB activator (TRAF)-binding kinase 1 (TBK1) and the inhibitor of NF-κB kinase (IKKi). The TBK1/IKKi complex phosphorylates and translocates transcription factor IRF3 into the nucleus, activating transcription of the interferon-β gene (15).

TAK1 also activates several MAPK pathways, including the ERK1/2, p38, and JNK MAPK pathways (15, 20, 21). MAPK activation requires signaling via a cascade containing at least three kinases. TAK1 serves as a MAPK kinase kinase (MAP3K) within the p38 and JNK pathways, which are crucial in terms of the generation of immune mediators (15, 21, 22). In addition, TAK1 regulates activation of COT/Tpl2, the only MAP3K that can activate MAPK kinases 1 and 2, leading to activation of the ERK1/2 pathway. This latter pathway plays roles in both the innate immune and inflammatory responses (23). All three MAPKs next phosphorylate downstream targets (including other kinases and transcription factors) that, in turn, regulate the transcription of many genes including those encoding proinflammatory cytokines (15, 21, 22). In this review, we will describe recent advances in our understanding of how miRNAs regulate the innate immune responses triggered by TLRs, which in turn activate the host immune defenses and inflammation. The roles played by miRNAs, the mechanisms by which miRNAs regulate components of TLR-signaling pathways, and the relevance of such actions in the context of host defenses and inflammatory disease will be discussed.

Overview of miRNA biogenesis

The biogenesis, roles played in target recognition and post-transcriptional gene regulation, and several other biological functions of miRNAs have been discussed extensively in many earlier reviews (24-26). Thus, we will summarize miRNA biogenesis only briefly.

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 all protein-encoding mRNAs, thus regulating genes of almost all biological processes. By base-pairing to mRNAs, miRNAs inhibit translation or increase mRNA turnover (25). To date, many miRNAs of mammalian cells have been identified and studied; miRNAs 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, and it is now becoming clear that aberrant miRNA expression is a general signature of many human diseases, including cancer, metabolic, and immune diseases (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 the 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), acting with the RNA-induced silencing complex (RISC) loading complex, processes 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 the duplex are separated by an RNA helicase (40), and one miRNA strand (the guide strand) of the duplex is loaded into an Argonaut-containing RISC (41, 42). Finally, the single-stranded mature miRNA pairs with mRNAs by interacting with the 3′ UTRs of those

mRNAs (43). This causes 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 activation of the innate immune system have been discussed extensively in several excellent reviews (11, 13). Thus, we will not visit these topics here. In our current review, we will briefly focus on miR-146a, miR-146b, miR-125a, and miR-125b and their regulation of innate immune, inflammatory, and antimicrobial responses (46-52).

i. MiR-146 and innate immune regulation

MiR-146a, a NF-κB-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 the inflammatory response (54). Earlier studies suggested that miR-146a is involved in linking the innate immune response to oncogenic transformation (55-57). MiR-146a plays a critical role in regulating the proliferation of immune cells and in inhibiting inflammatory responses (56, 57). An miR-146a deficiency in mice is associated with chronic dysregulation of NF-kB signaling, yielding a phenotype characterized by myeloid malignancy (57). Indeed, the gene encoding miR-146a (encoded on chromosome 5q33.3) has been reported to be absent in hematopoietic progenitor cells of many myelodysplastic syndrome (MDS) patients with 5q- syndrome (thus, with deletion mutations in a segment of chromosome 5q) (58).

Both miR-146a and miR-146b regulate inflammatory responses by targeting mRNAs encoding IRAK-1 and TRAF6 (46, 53, 59, 60). An in vivo deficiency in miR-146a triggers

macrophage hyperactivation, elevates the systemic response to endotoxin (lipopolysaccharide), and predisposes to the development of an autoimmune phenotype later in life (56). In addition, one study showed that miR-146a was characteristically upregulated in LPS-adapted human monocytic cells, suggesting that miR-146a might be a key regulator of endotoxin tolerance (61).

Recent in vivo studies using a lentivirus expressing miR-146a (LmiR-146a) showed that miR-146a was essential to prevent sepsis-induced NF-κB signaling and the generation of inflammatory cytokines and to inhibit IRAK and TRAF6 expression in the myocardium, thus attenuating the cardiac dysfunction often associated with sepsis (62). TLR3-stimulation of human nasal epithelial cells induced miR-146a synthesis via the PI3K, JNK, and NF-κB pathways (63). Notably, miR-146a played an important role in expression of the tight junction proteins claudin-1 and JAM-A, suggesting that miR-146a was essential for maintenance of the tight junction barrier and the innate immune defense (63). In primary human keratinocytes, miR-146a inhibited the development of NF-kB-dependent inflammatory responses by directly targeting recruitment (by the upstream nuclear factor kappa B) of three signal transducers; these were caspase domain-containing protein 10, IL-1 receptor-associated kinase 1, and CCL5 (64). Moreover, TLR2 stimulation triggered sustained expression of miR-146a, which in turn suppressed the synthesis of IL-8, CCL20, and TNF-α, in primary human keratinocytes (65).

In addition, activation of TLR4 signaling upregulated miR-146b expression in human monocytes via the action of an IL-10-mediated STAT3-dependent pathway (51). In turn, miR-146b negatively regulated LPS-mediated production of many proinflammatory cytokines and chemokines. MiR-146b fulfilled these roles by targeting many components of signaling pathways, including TLR4, the myeloid differentiation primary response protein (MyD88), IRAK-1, and TRAF6 (51). In human umbilical vein endothelial cells (HUVECs), prolonged expression of angiopoietin-1 significantly decreased LPS-induced IRAK1 and TRAF6 levels via upregulation of miR-146b-5p expression. Interestingly, angiopoietin-1 did not influence

the expression levels of miR-146a or miR-146b-3 in HUVECs (66). Together, the cited studies showed that both miR-146a and miR-146b serve as critical negative regulators of the activities of various cell types of the innate immune system, preventing the development of harmful inflammatory responses, and promoting the maintenance of homeostatic conditions.

ii. 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 and promotes IL-4-induced expression of the alternative M2 phenotype by targeting KLF13, a transcriptional factor active during T lymphocyte activation and inflammation (50). Additionally, miR-125a-5p suppressed the phagocytic and bactericidal activities associated with macrophage M1 functionality (50). Earlier studies showed that expression of both miR-125a-3p/5p and miR-146a were regulated at the transcriptional level after *Listeria monocytogenes* infection, which altered the miRNA profiles of host macrophages, although the precise functions of the affected miRNAs remain unclear (67). Our recent studies have shown that miR-125a-3p inhibits the antimicrobial responses to, and the host defenses against, mycobacterial infection by targeting the gene encoding the autophagy UV radiation-resistance-associated protein (68). Together, the data suggest that miR-125a may inhibit innate macrophage responses by regulating all macrophage differentiation, inflammation, and autophagy.

Interestingly, expression of miR-125b-5p (which has the same core sequence as miR-125a-5p) is modulated by NF-κB signaling; miR-125b-5p targets the 3'UTR region of the TNF-α gene to negatively regulate the inflammatory response (49). The expression of both miR-125b and miR-155 is negatively regulated by LPS-induced Akt1 activation; this regulated the extent of endotoxin tolerance/sensitivity in mice (69). In addition, LPS stimulation of human macrophages suppressed expression of miR-125b, but estradiol pretreatment eliminated this effect, enhancing the stability of κB-Ras2-encoding mRNA; κB-

Ras2 is a key inhibitor of NF-κB signaling (70). Recent data have also shown that miR-125b-5p directly targets and inhibits expression of the gene encoding 5-lipoxygenase, the key enzyme in the biosynthesis of leukotrienes that (critically) mediate both the innate immune and inflammatory processes (71). However, another study found that miR-125b promoted macrophage-mediated inflammation, increasing the expression of the co-stimulatory factor and enhancing antitumor activities by targeting IFN-regulatory factor 4 (72). The data thus suggest that miRNAs play (at least partly) 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 may serve as novel diagnostic or therapeutic targets. The relevance of miR-146a in disease contexts is now well-accepted. Early studies showed that miR-146a expression levels were significantly increased in peripheral blood mononuclear cells from rheumatoid arthritis patients, although the levels of TRAF6 and IRAK1, two targets of miR-146a, were similar between patients and healthy controls (73). MiR-146a expression levels were elevated in keratinocytes and skin disease lesions of patients with atopic dermatitis. In human primary keratinocytes, miR-146a inhibited the expression of many proinflammatory cytokines and chemokines including CCL5, CCL8, and ubiquitin D. In addition, miR-146a-deficient mice exhibited 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 the innate immune response and development of the clinical 5q-syndrome, a subtype of myelodysplastic syndrome (58). Recent studies have shown that triple deletion of the genes encoding TRAF-interacting protein, forkhead-associated domain B protein (TIFAB), and miR-146a increased the level of TRAF6 protein, sustained TRAF6-mediated signaling, and triggered hematopoietic dysfunction, explaining (in part) the pathogenesis of

high-risk MDS and acute myeloid leukemia associated with chromosome 5q deletions (74, 75). Recent studies have revealed that an interrelationship exists between miR-146a synthesis and the pathogenesis of hepatic injuries developing after hepatitis B virus infection (76). MiR-146a expression levels were upregulated in HBV-infected cells, infected mice per se, and human hepatitis B virus-infected patients. HBV X protein-induced NF-κB signaling was required for expression of miR-146a, which in turn downregulated 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 with miR-146a, much less is known about the roles played by miR-125a-5p in inflammatory disease. MiR-125a levels were significantly reduced in patients with systemic lupus erythematosus (SLE). MiR-125a overexpression inhibited the levels of regulated on activation, normal T-cell expressed and secreted (RANTES) inflammatory chemokines by controlling the expression levels of the predicted target gene (KLF13) (77). In addition, miR-125a-5p expression was reduced in active lesions of multiple sclerosis patients (78). Further analysis showed that miR-125a-5p was required to enhance the tightness of the brain endothelial barrier and to stimulate formation of thick cell-cell junctional complexes in the brain endothelium (78). Recently, miR-125a was shown to be essential in terms of regulatory T cell function (the miRNA suppressed expression of effector T cell factors). miR-125a levels were downregulated in peripheral CD4(+) T cells of patients with autoimmune SLE and Crohn's disease (79). miR-125b was reported to be of clinical relevance in patients with chronic eosinophilic rhinosinusitis and nasal polyps; miR-125b (the levels of which were elevated in patients) regulated the extent of inflammation in sinonasal mucosal samples from such patients (80). MiR-125b upregulation elevated interferon-β mRNA levels in airway epithelial cells, by targeting EIF4E-binding protein 1 (4E-BP1), thus exacerbating mucosal eosinophilia (80). Thus, miRNAs modulate the expression levels of genes involved in almost all cellular functions. Further exploration of the roles played by miRNAs in pathogenesis and

defenses against innate immune responses will yield miRNA-derived therapeutics useful to treat many human inflammatory disorders.

DISCUSSION

Our understanding of the roles played by miRNAs in innate immune signaling and regulation of inflammation has advanced rapidly and extensively over the past several years. Here, based on data from recent studies, we emphasize that both miR-144 and miR-125 are becoming recognized as important regulator miRNAs in the context of innate immune responses and play many roles in the regulation of such 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 and serve as important translational regulators in many human disease states, including inflammation and infectious disease. Unraveling of the miRNA network is important to allow us to understand the pathogenesis of, and develop potentially useful treatments for, many human inflammatory diseases. Accumulating basic research data on miR-146 and miR-125 have identified novel roles played by these materials and the molecular mechanisms involved. Such work will lead to the development of new diagnostic and therapeutic strategies for immune and inflammatory diseases.

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FIGURE LEGENDS

Figure 1. The levels of miR-146a and miR-146b are regulated by innate immune signaling. The miR-146a and miR-146b levels are up- or down-regulated by TLR-induced intracellular signaling. TLR3 activates PI3K-JNK and NF-κB signal pathways, which up-regulates the miR-146a level. TLR2 signaling activates the IRAK1-TRAF6-NFκB pathway that enhances inflammatory responses through production of proinflammatory cytokines and chemokines. The activation of miR-146a decreases proinflammatory cytokines via inhibition of 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.

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

Figure 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, up-regulated miRNA mediated chronic rhinosinusitis (CRS), Rheumatoid arthritis (RA), atopic dermatitis (AD), Myelodysplastic syndromes (MDS), acute myeloid leukemia (AML) and Hepatitis B virus infection (HBV). Lower, down-regulated miRNA mediated multiple sclerosis (MS), systemic lupus erythematosus (SLE), Experimental autoimmune encephalomyelitis (EAE), systemic lupus erythematosus (SLE) and Crohn's disease.

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Figure 1

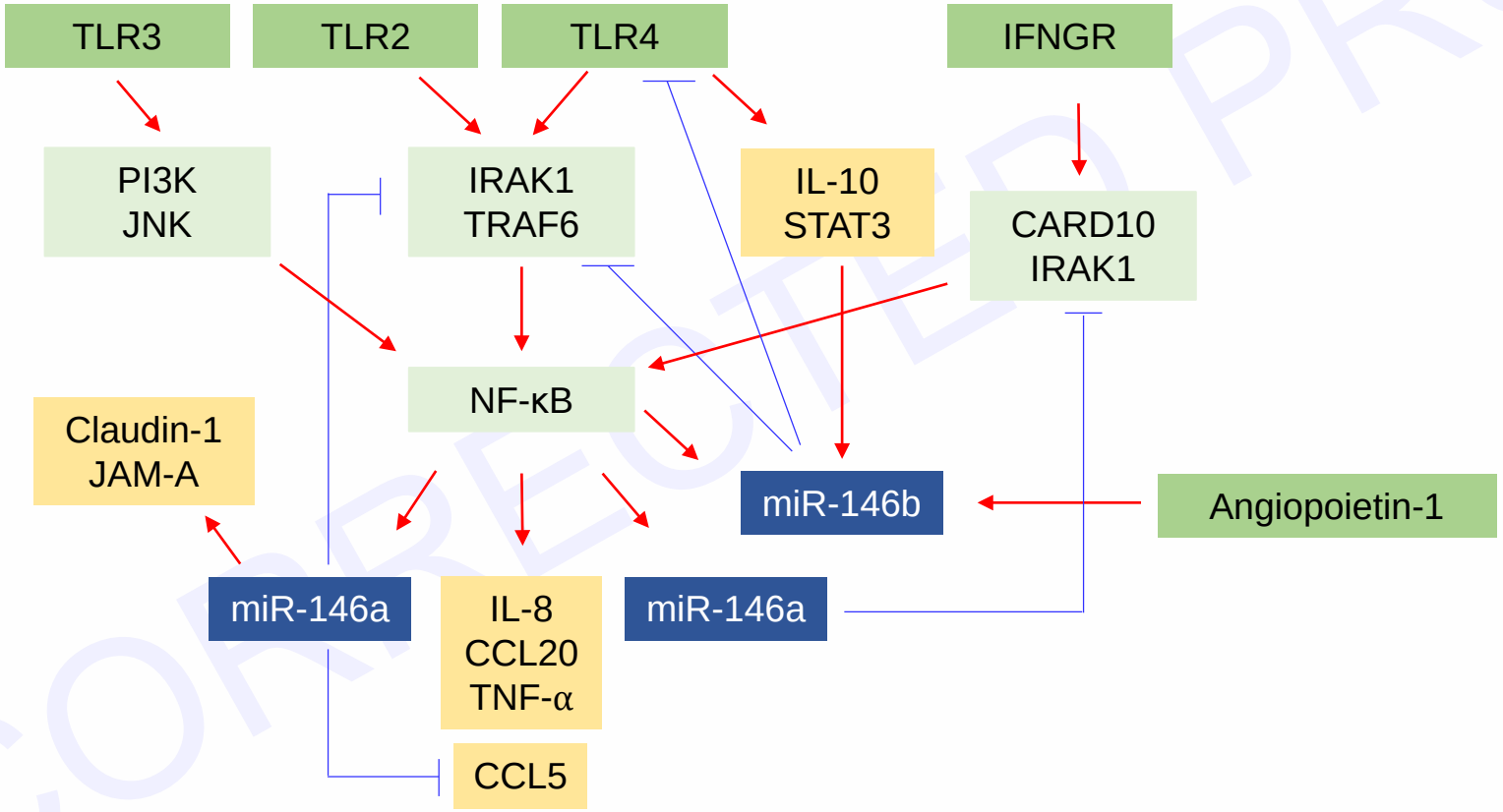

Figure 2

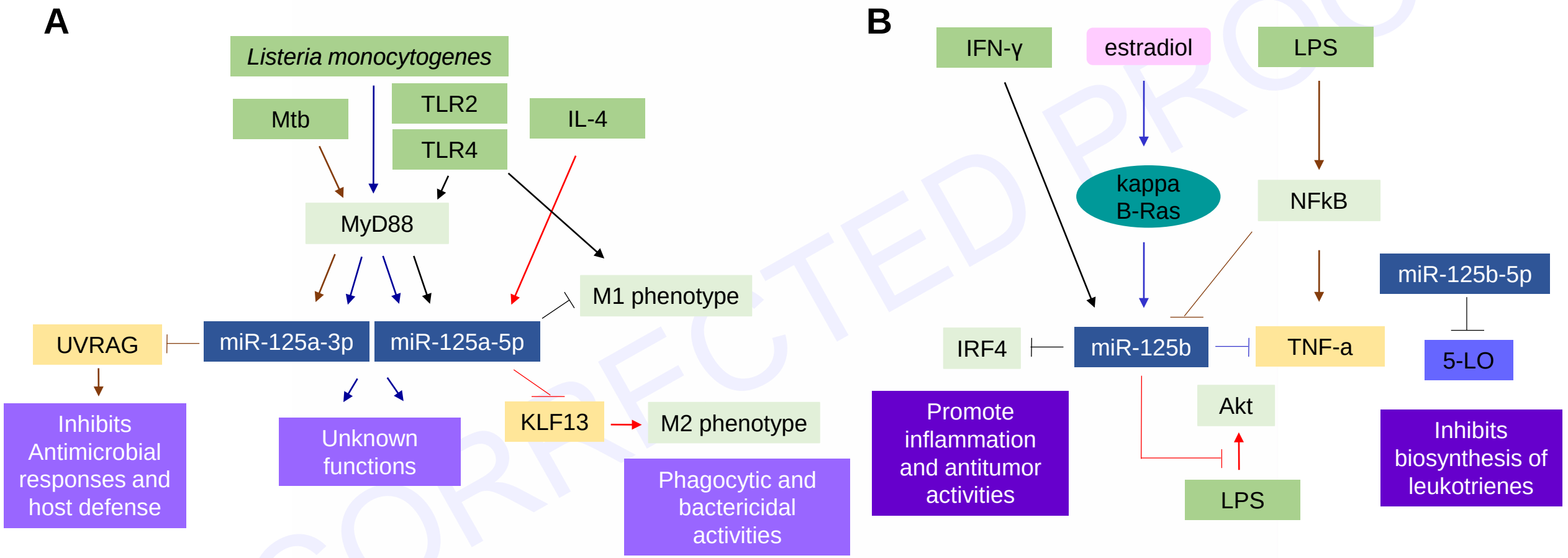

Figure 3