Role of MicroRNA in the Lung's Innate Immune Response

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Keywords

MicroRNA · Immune response · Respiratory host defense

Abstract

The immune response to respiratory pathogens must be robust enough to defend the host yet properly constrained such that inflammation-induced tissue damage is avoided. MicroRNA (miRNA) are small noncoding RNA which post-transcriptionally influence gene expression. In this review, we discuss recent experimental evidence of the contribution of miRNA to the lung's response to bacterial and viral pathogens.

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Introduction

The immune response to pathogens in the lung is tightly regulated, requiring numerous checks and balances to ensure adequate inflammatory signaling while not damaging host tissues via excessive inflammation. Cells use multiple mechanisms to regulate gene expression, including histone modification, DNA methylation, long-noncoding RNA expression and microRNA (miRNA) [1]. A key regulatory system capable of modulating most cellular pathways, miRNA are a family of small RNAs approximately 22 nucleotides in length that regulate gene

expression through the inhibition of mRNA translation and mRNA degradation [2]. MiRNA regulation of the immune system has been a topic of intense investigation [3, 4]. This review will discuss our current understanding of the contribution of miRNA to respiratory host defense against bacterial and viral pathogens. For a more in depth discussion of miRNA biology refer to the recent review articles by Valinezhad Orang et al. [5], and Ha and Kim [6].

Background on miRNA

miRNA are generally encoded in introns, and can be found in close proximity to the genes they regulate. While some miRNA share the same promotor as their target gene, this is not always the case. Therefore, identification of the miRNA target has been driven mostly by sequence-based prediction. Known miRNA and their target genes have been tabulated in the miRNA database miRBase [7, 8]. miRNA are transcribed from the genome by RNA Pol II, and possibly by viral Pol III, resulting in the production of primary miRNA, which are approximately 1 kb in size [9]. The nuclear RNase III Drosha then cleaves the stem-loop structure forming a hairpin-shaped (approximately 65 nucleotide) pre-miRNA [10]. The pre-miRNA then forms a complex with exportin 5 and GTP-binding nuclear protein RAN-GTP, which shuttles the pre-

miRNA into the cytoplasm where the GTP is hydrolyzed and the pre-miRNA is released. The final step in miRNA formation occurs here in the cytoplasm where it is cleaved by Dicer and incorporated into the RNA-induced silencing complex, where it interacts with its target RNA.

Host cells activate miRNA transcription following the recognition of pathogen-associated molecular patterns (PAMPs) by receptors such as the Toll-like family [11–14]. Downstream of PAMP receptors, signaling pathways, including molecules such as NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells), mitogen-activated protein kinases, signaling transducer and activator of transcription, regulate the transcription of miRNA along with their target genes [15–19]. The miRNA then act to limit the production of the target gene, acting as an internal break on gene transcription. This review focuses on recent findings on the contribution of miRNA to the response of host cells to respiratory pathogens.

miRNA in Specific Cell Populations

Epithelium

The airway epithelium, while not traditionally considered a component of the immune system, is uniquely positioned to significantly contribute to host defense against respiratory pathogens. The primary function of the epithelium is as a barrier separating “dirty” air from the “clean” blood stream. However, the epithelium is more than just a barrier. The epithelium is located such that it is constantly exposed to potential pathogens, and therefore has a critical role in initiating and coordinating host defense responses [20]. Its role in host defense is highlighted clinically by the increased infection rates of smokers and individuals with chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF), each associated with altered epithelial cell function [20–23].

miRNA are involved in all aspects of epithelial regulation. In order to maintain the physical barrier separating out from in, the epithelium is required to rapidly replace any dying or dead cells such that pathogens cannot cross the breach created by cell loss. During wound healing or mechanical stretch, the expression of a large number of miRNA is altered, suggesting a coordinated response to the physical stress through miRNA regulation [24, 25]. Epithelial renewal following injury is dependent on the transcription factor c-Myc, which in turn regulates a family of approximately 17 unique miRNA, including miR-126, miR-34c, miR-130a, miR-574, miR-193b, miR-19b,

miR-125b, miR-17, miR-214, and miR-34b [26]. These miRNA were predicted to target essential components of cellular proliferation, including polo-like kinase 4, tenascin C, and ubiquitin specific peptidase 1.

Epithelial wounding/healing is associated with the activation of signaling pathways, such as p38 MAP kinase and myosin light chain kinase, which promotes actin polymerization and epithelial regeneration [27–30]. Activation of this p38 in epithelial cells suppresses miR-17–92, therefore suppressing a negative regulator of lung development [31]. Two other key miRNA, miR-155 and miR-23a, are downregulated in epithelial cells infected with *Klebsiella pneumoniae*. These 2 miRNA act through high mobility group nucleosomal-binding domain 2 and nuclear factor 1 to regulate $\alpha 5 \beta 1$ integrin expression and actin polymerization at the cell surface, and to restrict *K. pneumoniae* adhesion [32].

The epithelium is also able to respond to pathogens by producing cytokines responsible for recruiting professional phagocytes such as neutrophils and by producing antimicrobial peptides to directly kill pathogens. miRNA are involved in both pathogen survival and innate immune function. The respiratory syncytial virus downregulates miR-221 in epithelial cells, reducing epithelial apoptosis and increasing viral replication, while influenza A downregulates miR-17-3p as well as miR-221, which also promotes viral replication [33, 34]. The downregulation of miR-276 during viral influenza A infection promotes epithelial apoptosis and viral clearance; therefore, a balance must exist to properly regulate the influence of miRNA on epithelial apoptosis. Pathogen killing is influenced by miRNA. For example, during influenza A infection, upregulation of miR-136 promotes IFN- β accumulation in an A549 epithelial cell model, promoting viral killing [35]. In response to bacteria such as *Mycobacterium bovis*, *Staphylococcus aureus*, or *Pseudomonas aeruginosa* altered expression of miR-21, miR-124, and miR-93 regulates production of inflammatory cytokines, such as IL-8, by the epithelium [36–38].

Macrophages

Respiratory macrophages, both resident alveolar macrophages and recruited inflammatory monocytes, have essential roles in the host defense against respiratory pathogens. Depletion of resident macrophages impairs host defense against a range of bacterial pathogens, including *S. aureus*, *K. pneumoniae*, and some *P. aeruginosa* isolates, as well as viral pathogens, respiratory syncytial virus and influenza A [39–43]. These cells are also

Fig. 1. Immune functions influenced by miRNA in the lung. NET, neutrophil extracellular trap; TLR, Toll-like receptor.

capable of promoting tissue damage during infections by pathogens such as ExoS+ *P. aeruginosa* and metapneumovirus [44, 45].

Macrophage binding to pathogens facilitates not only uptake and killing, but also antigen processing for the development of memory immune responses. Conversely, the attachment of viruses to the cell surface enables invasion of the cytoplasm and viral replication. Therefore, it is important that host cells are capable of tuning the expression of surface proteins involved in these processes such that phagocytic killing is enabled while viral replication is prevented. MiR-155 directly influences the phagocytosis of bacterial pathogens such as *P. aeruginosa* and *S. aureus* by modifying expression of the scavenger receptor MARCO [46]. Another scavenger receptor, CD163, enables the invasion and replication of PRRSV (porcine reproductive and respiratory syndrome virus) [47]. Alveolar macrophages suppress CD163 by upregulating miR-181 in response to this virus, thereby limiting viral replication [48]. Once the virus is internalized, miR-125b targets the NF- κ B to further limit PRRSV replication [49]. Therefore, by limiting surface binding and targeting signaling activated in response to internalized viruses, miRNA act to prevent the propagation of viral infection by limiting viral replication.

miRNA have been implicated in the regulation of multiple macrophage responses to respiratory pathogens. Toll-like receptors (TLR) are major receptors for pathogens, and they are directly influenced by miRNA [50]. MiR-124 targets TNF receptor-associated factor 6 (TRAF6), which is necessary for signaling downstream of TLRs [38]. Other miRNA alter the expression of signaling

regulators, such as dual specificity protein phosphatase 1 (DUSP1), targeted by miR-429, interleukin-1 receptor-associated kinase 4 (IRAK4), targeted by miR-302b, and phosphatase and tensin homolog (PTEN), targeted by miR-26b [14, 51, 52]. Macrophages also activate antimicrobial signaling pathways in nearby immune cells. One such pathway is the IL-23/IL-17 pathway shown to contribute to the host response to numerous pathogens [53, 54]. Multiple miRNA target this pathway, including miR-146 and miR-155 [55–57]. These miRNA are therefore able to regulate the alveolar macrophage's response to multiple pathogens by influencing proteins central to multiple signaling pathways.

Neutrophils

Neutrophils are the most abundant of the immune cells, estimated to comprise up to 80% of the total immune cell population, although in an uninfected host most of these cells reside in lymphoid tissue, such as the bone marrow and spleen, or are adherent to the vascular endothelium (marginating pool). Primarily studied in the context of bacterial and fungal infection, neutrophils are essential for proper defense against most pathogens [39, 58, 59]. Clinical diseases such as chronic granulomas disease are associated with defects in neutrophil function, and as such these patients are highly susceptible to infection, especially with bacterial (*S. aureus*, *Klebsiella* species, *P. aeruginosa*) and fungal (*Aspergillus* and *Candida* species) pathogens [60]. While the role of neutrophils is less studied in the context of respiratory viral infection, it is clear that neutrophils also play a protective role in a variety of viral pneumonias [61–63].

Table 1. Contribution of miRNA to cellular immune responses

Cell	miRNA	Function	Reference
Epithelium	miR-17	Proliferation	26
	miR-19b		
	miR-34b		
	miR-34c		
	miR-125b		
	miR-126		
	miR-130a		
	miR-193b		
	miR-214	Actin polymerization/ lung development	31
	miR-574		
	miR-17-92	Actin polymerization/ integrin expression	32
	miR-23a		
	miR-155	Apoptosis	33
	miR-221		
	miR-17-3p	Apoptosis/ viral replication	34
	miR-221		
	miR-276	IFN- β	35
	miR-136		
Macrophage	miR-155	Phagocytosis	46
	miR-181	Viral internalization	48
	miR-125b	Viral replication	49
	miR-124	TRAF6/TLR signaling	38
	miR-429	DUSP1	14
	miR-302b	IRAK4	51
	miR-26b	PTEN	52
	miR-146	IL-23/IL-17	55–57
Neutrophil	miR155	Chemokine production	64
	miR-223		
	miR-223	Granulocyte proliferation	65
	miR-146a	Antimicrobial processes/ phagocytosis, reactive oxygen species	66, 67
	miR-328		

Regulation of neutrophil function is required to prevent neutrophil-mediated host damage. As part of their antimicrobial arsenal, neutrophils release multiple pro-inflammatory mediators including cytokines, proteases, and DNA. If left unregulated overwhelming inflammation can result in tissue damage. miRNA play a central role in neutrophil regulation. Neutrophil recruitment is influenced by miR-155 and miR-223, which reduce chemokine production in response to pathogens [64]. miR-223 is also able to prevent granulocyte proliferation, limiting the numbers of neutrophils available to respond to

infection [65]. Antimicrobial function is directly influenced by miRNA. miR-146a and miR-328 negatively regulate neutrophil elastase, phagocytosis, and reactive oxygen species production, limiting the killing capacity of the neutrophil [66, 67]. Neutrophil extracellular traps, or extruded DNA used by neutrophils to trap and kill pathogens, are in part driven by IFN- γ . This process is regulated by miRNA, although the specific miRNA have yet to be determined [68]. Loss of miRNA regulation of neutrophil function therefore has the capacity to severely impair host defense in the lung.

T Cells

T cells and natural killer T cells have been shown to both protect against respiratory infection as well as contribute to inflammatory tissue damage during such infections. These cells contribute to both the innate response and adaptive immune response to respiratory pathogens, and therefore it is highly likely that miRNA contribute to the regulation of these cell populations. Currently, there is a paucity of data directly testing this hypothesis; however, a handful of studies have looked at miRNA regulation of these cells in the context of other respiratory diseases which are commonly associated with infection. The IL-22/IL-17 signaling axis can promote viral clearance when properly regulated, but high expression levels, or activation of this pathway, can result in inflammatory tissue damage [69, 70]. Cellular regulators such as miRNA are therefore uniquely positioned to regulate this pathway, and in fact miR-323-3p, miR-19, and miR-22 have been shown to alter the IL-17 pathway in the lungs of smokers and subjects who suffer allergic reactions and asthma [71–73]. Therefore, while not directly tested, there is evidence to suggest that miRNA regulate natural killer and T cell function during respiratory infection.

miRNA in Inflammatory Respiratory Disease

Pulmonary diseases such as COPD and CF are associated with pathogen-induced exacerbations or chronic infection [12, 21, 74]. There is mounting evidence that miRNA expression is altered in these diseases, potentially impairing immunity against pathogens in the lung; however, few studies have been published describing the role of individual miRNA in these diseases [75–77]. Microarray analysis of COPD lung samples identified miRNA-218-5p as being downregulated in the airway epithelium [78]. Using a smoke-induced COPD murine model, Conickx et al. [78] showed that a miR-218-5p mimic protected against smoke-induced inflammation, confirming

its role in COPD-associated inflammation. In macrophages, the autophagy process has a central role in the response to phagocytosed pathogens, and miRNA have been identified as key regulators of autophagy [79–82]. Recent work has identified a cluster of miRNA, miR17-92, that is elevated in CF macrophages [83]. These miRNA negatively regulate the autophagy pathway and CFTR expression, impairing host response to CF-related pathogens such as *Burkholderia cenocepacia*. Altered miR-31 in CF epithelial cells also contributes to the chronic inflammation associated with this disease [37]. Reduced levels of miR-31 results in the uncontrolled production of cathepsin S, which degrades essential antimicrobial peptides such as lactoferrin and β -defensins. Loss of these antimicrobial peptides makes the lung more susceptible to colonization with pathogens such as *P. aeruginosa*. Targeting miRNA in a therapeutic setting presents an exciting opportunity to correct some of the immune defects associated with these respiratory diseases [84].

Conclusions

miRNA act as key regulators of multiple cellular processes; however, testing their contribution to pulmonary immunity has only recently begun. Multiple cell types contribute to host defense in the lung, and miRNA regulate processes in each, including epithelial cells, macrophages, neutrophils and T cells, among others (Table 1; Fig. 1). Our understanding of how individual miRNA are regulated and which genes they target is still being developed, which has hampered the targeting of miRNA in the clinic. The next phase of miRNA development for clinical application involves not only more detailed analysis of miRNA biology, but also the invention of a methodology to target miRNA modulators to specific cell types. The ability to tailor miRNA therapy such that their capacity can be harnessed in a cell-specific and regulated manner has the potential to benefit patients suffering from a myriad of respiratory infections.

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