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MicroRNAs in Lung Disease

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ABSTRACT

Chronic inflammatory diseases of the lung are often life-threating and are a leading cause of morbidity in our communities. MicroRNA (miR) are now recognized to play critical roles in a wide-range of cellular functions including the regulation immunological processes, which are often dysregulated in chronic respiratory diseases. These small non-coding RNA molecules regulate networks of genes by inhibiting translation by targeting one or multiple messenger RNA transcripts. In this review we highlight discoveries that identify important roles for miRs in the regulation of specific pathogenic features of a range of diseases. Further, experimental evidence suggests that pharmacological inhibition miR function or deliver of mimics may have therapeutic potential. Thus we also discuss the potential utility and limitations of therapeutically targeting these molecules and their downstream pathways.

Keywords: respiratory diseases; miRNAs; immunological responses

Abbreviations: AHR = airway hyperresponsiveness; ALI = acute lung injury; BPD = bronchopulmonary dysplasia; CCL = C-C motif chemokine ligand; COPD = chronic obstructive pulmonary disease; CXCL = C-X-C motif chemokine ligand ; EMC = extracellular matrix; EMT = epithelial-mesenchymal transition; HDM = house dust mite; IFN = interferon; IL = interleukin; IPF = idiopathic pulmonary fibrosis; IV = influenza virus; LPS = lipopolysaccharide; miR = miRNA; NF-kβ = factor nuclear kappa β; OVA = ovalbumin; PBMC = peripheral blood mononuclear cells; RSV = respiratory syncytial virus; RV = rhinovirus; TGFβ1 = transforming growth factor-β1; TNFα = tumor necrosis factor α.

MICRORNAs

MicroRNAs (miRs) are small non-coding RNA molecules (~22 nucleotides in length) that inhibit protein translation in cells by blocking mRNA access to the translational machinery or by directly causing degradation of mRNA transcripts [1]. MiRs can regulate multiple mRNAs by binding to the complementary sequences of the mRNA (in their 3' untranslated region). These miR-mRNA interactions are facilitated by base pair complementary binding of the seed sequence of miRs, located between nucleotides 2-8 at the 5' end of the miRs. Later studies demonstrated that imperfect binding of the miRs seed regions was also tolerated and can be compensated by pairing of the miRNA 3' end to the mRNA transcript [1-3]. The canonical and non-canonical binding modes of miRs are further reviewed by Hausser *et al.* [3]. These unique miR-mRNA interactions mean that miRs dysregulation can affect multiple genes and potentially have functional implications on several pathways that contribute to disease pathogenesis. MiRs to date was known to play critical roles in regulating wide-range of cellular functions and regulatory pathways such as apoptosis, organ development, cell differentiation and proliferation (please see reference [4] for a comprehensive description). Over the last ten years, as the biological role of miRNAs have been discovered, their dysregulation has been linked to the pathogenesis of a range of diseases. In this review we will highlight key findings that demonstrate the importance of these small RNA molecules to the

regulation of cells that are found in the lung and their contribution to the mechanisms underpinning respiratory disease.

ASTHMA

Asthma is a chronic inflammatory disease of the lungs characterized by the influx of a range of activated leukocytes (e.g. eosinophils and neutrophils) that often correlates with clinical features of disease such as airway hyperresponsiveness (AHR), airflow obstruction, mucus hypersecretion, and structural alterations of the airway wall (remodelling) [5]. A growing number of studies have demonstrated significant alterations in the expression and functional roles of miRs, which are linked to pathogenic processes [6].

Interleukin (IL)-13 and the transcription factor signal-transducer-and-activation-of-transcription-6 (STAT6) operated pathways have been shown to play critical roles in the regulation of hallmark clinical features of asthma (e.g. AHR and remodelling) [7]. In this regard, miR-155 has been shown to be upregulated to directly target transcripts for the IL-13 receptor α1 (IL13Rα1) in human macrophages, reducing the levels of IL13Rα1 protein, decreasing levels of activated STAT6, which plays a pivotal role in regulating the IL-13 signalling pathway [8]. In conjunction with IL-13, other genes that regulate inflammation have been described to be regulated by miR-155, such as suppressor of cytokine signalling 1 (SOCS1), C-C motif chemokine ligand (CCL)-18 and Fc epsilon RII (CD23) [8]. These molecules have been implicated in the pathogenesis of asthma and allergic diseases [8]. The expression of miR-125b was decreased in asthmatic sputum samples by comparison to controls and this miR was shown to regulate SAM-pointed-domain-containing-ETS-transcription-factor (SPDEF), which plays a crucial role in the differentiation of airway goblet cells [9]. Moreover, when miR-125b was overexpressed in the lungs during the development of allergic inflammation in a house dust mite (HDM) mouse model of asthma, mucus hypersecretion and goblet cell differentiation were significantly decreased [9].

Mouse models have been widely used for the study of asthma pathogenesis and are often based on sensitisation and aeroallergen challenge with Type-2 inducing allergens such as ovalbumin (OVA) and HDM. Deep sequencing and microarray analysis have demonstrated increased levels of the miR-let-7 family in an OVA-induced model of allergic asthma [10]. Inhibition of miR-let-7 also decreases the levels of a range of inflammatory cytokines (e.g. the type-2 cytokines IL-4, IL-5 and IL-13), revealing a proinflammatory role of these miRs [10]. The use of a miR-145 inhibitor in a model of HDM induced asthma decreased the levels of IL-5, IL-13, eosinophils and goblet cell hyperplasia in the lung [11]. Similarly, the levels of miR-16, miR-21 and miR-126 were also increased by HDM exposure when compared to controls and shown to regulate key disease features [12]. For example, both miR-126 and miR-145 were shown to be involved in regulation of inflammation (e.g. eosinophilia), AHR, mucus hypersecretion and cytokine production by T helper cell type 2 (Th2 cells) (through reduced production of IL-5 and IL-13) [11, 12]. Expression of miR-126, was regulated by the toll-like receptor-4 (TLR4) and myeloid-differentiation-primary-response-88 (MyD88) pathways [12]. In addition, Collison *et al.* [11] have demonstrated that the use of antagomir (ant) to inhibit miR-145 function also inhibited IL-5 and IL-13 by Th2 cells, decreased the number of eosinophils recruited to the airways, mucus hypersecretion and the development of AHR, results that were shown to be similar as to steroid treatment (a primary clinical treatment) [11].

MiRs have also been described to play a role in steroid-resistant asthma, which affects 5-10% of patients [13]. MiR-9 levels were found to be increased in the sputum of neutrophilic asthmatic patients (non-type 2) that are often resistant to the glucocorticoid therapy [14]. In the same study, using a mouse model of steroid-resistant asthma, miR-9 was found to be increased in airway

macrophages and lung tissue [14]. Inhibition of miR-9 function reduced AHR and restored the anti-inflammatory effects of dexamethasone by increasing the activity of the miR-9 target, protein phosphatase 2A (PP2A) [14]. In infection (e.g. *Haemophilus influenzae*, influenza (IV) and respiratory syncytial virus (RSV)) triggered exacerbation models of asthma, steroid-insensitive inflammation and AHR in the lung were regulated by miR-21 modulating the PI3-kinase-mediated pathway [15]. Inhibition of miR-21 restored dexamethasone sensitivity resulting in attenuation of Th2 cytokine levels (IL-4, IL-5 and IL-13), numbers of infiltrating airway inflammatory leukocytes and AHR [16]. Collectively, these studies show that targeting miRs directly or their downstream targets may provide novel anti-inflammatory approaches to attenuate inflammatory responses in asthma [12].

IDIOPATHIC PULMONARY FIBROSIS (IPF)

IPF is a chronic and progressive disease of the lungs characterized by dense fibrosis and increased proliferation of fibroblasts [17]. Epithelial injury in IPF is thought to initiate a series of events, including upregulation and downregulation of miRs, which may regulate the development of fibrotic lesions [18]. Indeed, microarray analysis of lung samples from IPF patients and healthy controls indicates a significant difference in the expression of miRs between diseased and healthy individuals, suggesting that miRs play an important role in pathogenesis [18].

In IPF, transforming growth factor-β1 (TGF-β1) is released in response to injury of lung tissue, which stimulates fibroblasts differentiation to promote wound healing [18]. MiR-let-7d and miR-26 are both decreased in IPF compared to healthy lungs, and play critical roles in regulating epithelial-mesenchymal transition (EMT) [18]. MiR-let-7d was shown to be negatively regulated by TGF-β1 expression (via binding of the transcription factor Small-mother-against-decapentaplegic-3 (Smad3) to the miR-let-7d promoter) in alveolar epithelial cells from IPF patients [18]. In preclinical models of IPF a decrease in miR-let-7d function results in the upregulation of high-mobility group-A2 protein (HMGA2), which promotes EMT and increases deposition of collagen [18]. Similarly, miR-26a was shown to be downregulated in lungs of IPF patients and in an experimental model of IPF [19]. MiR-26a can bind to the 3' untranslated region of TGF-β1 and HMGA2 transcripts to repress their protein translation [20]. Thus, downregulation of miR-26a expression in IPF increases TGF-β1 and HMGA2 levels, which subsequently promotes EMT and pulmonary fibrosis [20].

The role of miR-29 in IPF has also been extensively investigated [21, 22]. For instance, miR-29 targets production of extracellular matrix (ECM) related proteins such as elastin, fibronectin and collagens, suggesting that this miR has regulatory effects on ECM deposition and production [23]. Due to its downregulation in IPF, mimics of miR-29 were employed and it was observed that increased levels of miR-29 had antifibrotic effects in a bleomycin induced mouse model of fibrosis [24]. Interestingly, these effects lasted for days suggesting that mimics may have therapeutic applications [24]. Herrera, J., *et al*. (2018) investigated how the ECM may also regulate miR-29 expression in myofibroblasts [25]. They demonstrated that the ECM supressed Dicer1 expression, thus inhibiting the processing of the precursor of miR-29, resulting in decreased miR-29 levels and inhibition of its anti-fibrotic action [25]. MiR-708-3p was also shown to be downregulated and may also play a role in regulating fibrosis in IPF [26]. MiR-708-3p directly targets ADAM metallopeptidase-domain-17 (ADAM17) to affect the GATA binding protein/signal-transducer-activator-of-transcription 3 (GATA/STAT3) signalling pathways [26]. GATA-3 was shown to play a central role in Th2 polarization and cytokine production. IL-4 and IL-13 were demonstrated to enhance fibrotic processes by increasing proliferation of fibroblasts and production of collagen [27].

CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

COPD is a term used to describe diverse and progressive disorders of the lung characterized by pronounced inflammation and destruction of the parenchyma, which results in limitation of airflow. Tobacco smoking is one of the major risk factors for COPD [28]. Expression profiling of miRs in lung samples from COPD patients and from individuals with normal lung function demonstrated that the majority of miRs were downregulated in COPD patients, suggesting widespread disruption in regulatory control of these non-coding species [29].

MiRs can also be detected in blood samples from COPD patients. For example, levels of miR-146a/b were shown to be downregulated and negatively correlate with the levels of expression of inflammatory mediators such as tumor necrosis factor α (TNF-α), IL-6, IL-8, leukotriene E4 (LTE-4) and leukotriene B4 (LTB-4) in patients with acute exacerbation of COPD [30]. This study suggested that miR-146a/b may be a promising biomarker to predict the risk of acute exacerbations in stable COPD patients [30]. Similarly, miR-218-5p plasma levels are downregulated in COPD patients compared to control subjects [31]. Inhibition of miR-218-5p in experimental models of COPD results in increased numbers of neutrophils, dendritic cells and T-cells, suggesting an anti-inflammatory role for this miR [31]. In another study, miR-195 was found to be increased in lung tissues from COPD patients [32]. PH-domain leucine-rich-repeat-protein-phosphatase-2 (PHLPP2), which is a negative regulator of Akt serine-threonine kinases, was found to be a direct target of miR-195 [32, 33]. Hence, an increase in miR-195 expression will enhance Akt signalling that may contribute to the persistence of inflammatory responses in COPD [33]. In an experimental model of cigarette smoke (CS) induced COPD, treatment with anti-miR-195 lentivirus construct alleviated CS-induced lesions and inflammation in the lung [32].

ACUTE LUNG INJURY (ALI)

ALI is a severe respiratory disease characterized by acute inflammation, production of proinflammatory mediators and destruction of the epithelial and endothelial barrier of the lung [34]. Recent studies demonstrate that miRs play pathogenic roles in mouse models of lipopolysaccharide (LPS)-induced ALI [35]. In this model, miR-125b was found to be downregulated in the serum after LPS exposure [35]. Interestingly, miR-125 levels are also decreased in peripheral blood samples taken from patients with acute respiratory distress syndrome (ARDS) – a severe form of ALI [36]. Further studies demonstrated that the overexpression of miR-125b inhibits weight loss, decreases lung inflammation and increases survival of mice following LPS exposure [36]. These findings may be explained by the fact that miR-125b targets TNF-α transcripts thereby inhibiting its protein translation [37]. The overexpression of miR-454 also supresses the severity of LPS induced ALI by decreasing levels of C-X-C motif chemokine ligand (CXCL) 1, CXCL2, IL-6 and TNF-α [38]. MiR-454 was also shown to inhibit translation of the chemoattractant, CXCL12, in human lung epithelial cells [38].

Further investigations in a mouse model of ALI have shown increased expression of the transcription factor forkhead-box-A1 (FoxA1), along with a significant downregulation of miR-17 [39]. Inhibition of miR-17 function using an anti-sense antagomir resulted in the increased expression of FoxA1 [39]. This transcription factor is known to promote cell growth and differentiation in a variety of organs, including the lung [39]. Yuan, Z., *et al*. (2016) have also implicated triggering-receptor-expressed-on-myeloid-cells-1 (TREM-1), a super-immunoglobulin receptor, in the pathogenesis of LPS induced-ALI [40]. TREM-1 promotes inflammation by increasing miR-155 expression, which downregulates SOCS-1 protein translation [40]. The inhibition of TREM-1 function resulted in a decrease of miR-155 expression in lung and in macrophages after exposure to LPS [40]. In this study, levels of neutrophils and cytokines/chemokines (e.g. IL-6, IL-1β and TNF-α) were also decreased after TREM-1 inhibition

[40]. The proinflammatory role of TREM-1 was inhibited by the blockade of miR-155 function with antagomirs further demonstrating the relationship between these two molecules for the regulation of inflammation [40]. MiR-429 has also been known to regulate inflammation by directly targeting a negative regulator of proinflammatory cytokine production, the dual-specificity phosphatase 1 (DUSP1) [41]. Following LPS stimulation, DUSP-1 is able to inactivate p38 mitogen-activated protein kinase (p38 MAPK) signalling and subsequently decreases the production of IL-1β, IL-6 and TNF-α [41]. In a proof-of-principle study, treatment with anti-miR-429 inhibitor resulted in reduced inflammation (TNF-α, IL-1β and IL-6) in a rat model of ALI [41]. Xie, W., *et al*. (2018) have also demonstrated that miR-34b-5p, a miR mainly expressed in the lungs, targets progranulin (PGRN) production [42]. PGRN is expressed in macrophages and in epithelial cells and is involved in negative regulation of a range of processes regulating inflammation, apoptosis and wound healing [42]. Inhibition of the expression of miR-34b-5p upregulated PGRN, which subsequently resulted in the attenuation of inflammation in the lungs (e.g. decreased levels of TNFα, IL-6 and IL-1β) and apoptosis in an LPS-induced ALI mouse model [42]. Epithelial apoptosis is one of the events that is often associated with ALI. MiR-181a was shown to promote apoptosis in human epithelial cell lines in response to LPS treatment and an inhibition of miR-181a function protects mice from LPS-induced ALI by upregulating the anti-apoptotic factor, B-cell lymphoma-2 (BCL-2) [43]. BCL-2 impedes mitochondrial release of cytochrome C and therefore supresses caspase-3 activation to inhibit apoptosis [43]. This study demonstrated the role of miRs in regulating epithelial cell death during ALI [43].

BRONCHOPULMONARY DYSPLASIA (BPD)

BPD is a lung disease that affects newborns and infants [44]. It is caused by long-term use of oxygen therapy and also by the use of mechanical ventilation [44]. When analysing histology samples, the disease is characterised by fibrosis, unusual elastin deposition and capillary growth, low alveolarization levels and mesenchymal cell hyperplasia [44]. Examination of lung samples from neonatal infants who died of BPD demonstrated that the expression of the miR-17˜92 cluster [45] was lower in these patients, by contrast to neonates that died from diseases not related to the respiratory tract [46]. Moreover, the investigators provide evidence that the miR-17~92 cluster, known to be involved in normal lung growth and development [46], may be a biomarker to predict BPD development [46]. Similarly, miR-206 was also shown to be downregulated in human samples and mouse models of BPD, and may potentially contribute to the progression of disease by increasing fibronectin 1 (FN1) production [47]. FN1 levels are elevated in both BPD tissue and in preclinical mouse models [47]. Downregulation of miR-206 may result in the upregulation of FN1, which can then drive extracellular matrix remodelling as BPD progresses [47]. In another investigation, Lal, C. V., *et al*. (2018) analysed tracheal aspirates from preterm neonates and identified miRs that could be predictive of the severe form of BPD [48]. MiR-876-3p expression levels were reduced the most in these samples, and thus may be a biomarker for BPD [48]. Similar findings were found in *in vivo* and *in vitro* models of BPD. Notably, gain-of-function of miR-876-3p was shown to ameliorate the alveolar destruction in a mouse model of BPD [48]. To date, there are few miRs that have been studied in relation to the pathogenesis of BPD.

VIRAL INFECTIONS

Viral infections such as rhinovirus (RV), respiratory syncytial virus (RSV) and influenza virus (IV) are the main triggers of exacerbation of many respiratory diseases (e.g. asthma and COPD). MiRs are known to regulate both innate and adaptive immune responses and host antiviral immunity [49, 50].

Ouda, R., *et al*. (2011) demonstrated that miR-23b inhibits the minor group of RV (RV1B) infection by downregulating the very-low density lipoprotein (VLDL) receptor, which facilitates RV1B entry into cells [51]. By contrast, miR-128 and miR-155 were discovered to directly target RV1B RNA and inhibit viral replication [52]. Coincidentally, these miRs were also found to be downregulated in the epithelium of asthmatic patients, which often have impaired anti-viral responses [52].

RSV infection has been associated with altered expression of miR-let-7b, miR-let-7i, miR-30b and miR-221 in a cell-type-specific manner (e.g. members of miR-let-7 target IL-6 and RSV induces the secretion and expression of this cytokine in macrophages) [53-55]. In a separate study, miR-146a-5p (upregulated) and miR-let-7c-5p, miR-221 and miR-345-5p (downregulated) expression was found to be altered in the human epithelial cell line (HEp-2), which were persistently infected with RSV [56]. The functional roles of these miRNA in RSV infection remain to be investigated.

Profiling studies between healthy and IV-A infected patients reported dysregulation of 193 miRs, independently of the stage of the infection [57]. Downregulation of miR-302a was found to correlate with an increased expression of interferon regulatory factor-5 (IRF5) in throat swab samples and in peripheral blood mononuclear cells (PBMCs) from infected patients [58]. Further investigations demonstrated that increased levels of miR-302a (by using mimics) downregulates IRF5 expression [58]. This consequently reduces the production of TNFα, interferon β (IFNβ), CCL-2, CCL-5, IL-6 and IL-8 in PBMCs infected with IV, leading to increased viral replication [58]. MiR-4776 was found to target the regulatory protein for factor nuclear kappa β (NF-kβ), factor nuclear kappa β inhibitor β (NFKBIB), which plays a critical role in apoptosis, cell proliferation and survival [59]. Downregulation of this regulatory protein by miR-4776 resulted in increased NF-kβ production, which may regulate IV survival [59] (For a comprehensive review on the regulation of the antiviral responses to IV by miRs see [60]).

BACTERIAL INFECTIONS

Similar to viral infections, pathogenic bacteria often infect susceptible individuals that have chronic respiratory inflammatory diseases and are associated with exacerbation of these disorders (e.g. asthma and COPD). MiRs are also being increasingly implicated in the mechanism of host defence against microbial infection. [61].

For example, we have demonstrated that miR-328 expression was regulated by p38 and c-jun N-terminal kinases (JNK) signalling pathways, and that inhibition of miR-328 *in vitro* resulted in increased non-typeable *Haemophilus influenzae* (NTHi) clearance by neutrophils and macrophages by enhancing phagocytosis [62]. Furthermore, the use of an antagomir-328 *in vivo* resulted in accelerated NTHi clearance in mouse models of cigarette-induced (CI) emphysema and steroid-induced immunosuppression [62]. MiR-23a-5p has been shown to promote *Mycobacterium tuberculosis* survival in macrophages and inhibit the induction of autophagy of these infected cells [63].

MiRs have also been demonstrated to be involved in post-viral secondary bacterial infection. For example, miR-155 expression was found to be elevated in alveolar macrophages isolated from IV-A infected patients with secondary bacterial pneumonia [64]. Similarly, mice infected with IV followed by methicillin-resistant *Staphylococcus aureus* (MRSA) infection demonstrated a further increase in miR-155 expression [64]. Inhibition of miR-155 with antagomirs increased IL-23 and IL-17 production and improved bacterial clearance in lungs [64]. These studies demonstrated that miRs function in macrophages can be manipulated to enhance bacterial clearance.

POTENTIAL THERAPIES

An increasing number of miRs are being shown to play critical roles in a range of respiratory disorders (Table 1). MiRs can regulate entire signalling networks that are central to pathogenic process (Figure 1), which make them promising as novel therapeutics (mimics) or by pharmacological inhibition. Specific miR and subsets are also indicative of severity and types of disease making them potential biomarkers that are diagnostic and prognostic.

One of the challenges of regulating miR function is targeting specific miR in the diseased organ and potential side-effects. Therapeutic application needs to be approached with caution as any given miR has multiple targets and its function in different cells may alter dramatically. Of target effects may predispose to cancer, alterations in immunity and other cellular abnormalities (e.g. alterations in metabolism). Another challenge of delivering miR (mimetics and inhibitors) as therapeutics is degradation by nucleases before modulation of targets, although their stability and half-life *in vivo* are greatly enhanced by employing chemically-modified oligonucleotides [65].

However, individual miRs or clusters are often transcribed in the context of highly specific transcriptional programs initiated by a specific stimulus (e.g. infection, cytokine storms and pathogenic factors). Under these conditions, direct targeting of critical miRs, or importantly their targets which drive disease, may be of therapeutic value. In the past few years pharmaceutical companies have started clinical trials for miRNA-based therapies. For example Santaris Pharma have developed Miravirsen (SPC3649) with the aim to inhibit miR-122 function for the treatment of hepatitis-C virus infection. In phase-II clinical trials, a locked nucleic acid phosphorotioate-modified oligunocleotide (complementary to the 5' end of miR-122) (Miravirsen) was administered intravenously and this resulted in the suppression of viremia [66]. Modulation of miR-34 function is also being explored for cancer therapy. MiR-34 acts as a tumour suppressor in several types of cancers by regulating the p53 pathway by targeting oncogenes (MYC, MET, BCL2, β-catenin). However, a phase I clinical trial on liver cancer was halted due to immunological adverse effects [67]. An alternative approach may be to identify the critical targets of miR-34 and develop agents to modulate these downstream factors.

The transition from bench to bedside for miRNA-based therapy remains to be determined. In respiratory disease, a miR-based drug can potentially be administered locally (via inhalation or intranasal route) to modulate disease. Direct administration of a miR-based drug into the lungs may result in enhanced bioavailability, lower delivery dose and reduced side effects. The successful clinical application of miR-based therapy for lung diseases will largely be dependent on the identification of promising candidate miRs and the factors they regulate. In this regard, a full characterisation of the downstream miRs targets and their functions are required. In addition to this, the development of suitable carriers that allow cellular-specific uptake of miR-based drugs is also critical to enhance efficacy and limit toxicity.

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Table 1. MiRNAs function in respiratory disease

Figure 1. MiRNAs regulate signalling network in lung. MiRNAs can modulate signal transduction pathway by inhibiting transcript translation. Exposure to stimuli such as allergens, infectious agent, smoke particulates, and reactive oxygen species (ROS) initiate a signalling cascade that promote inflammatory responses, cell survival and alteration in miRNAs expression. In lung, upregulation of pro-inflammatory miRNAs and downregulation of anti-inflammatory miRNAs can both promote inflammation and contribute to the pathogenesis of lung disease. Similarly, miRNAs can regulate cell survival pathway and contribute to lung injury and fibrosis.

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Disease	Expression in disease	miRNA	Function	Target	Reference
ASTHMA	up	miR-155	Regulates IL13Rα1 protein	IL-13Rα1	[8]
	up	miR-let-7	Regulates IL-13	IL-13	[10]
	up	miR-145	Regulates Th2 allergic inflammatory responses	Unknown	[11]
	up	miR-16, -21, -126	Regulates Th2 allergic inflammatory responses	Unknown	[12]
	up	miR-9	Regulates PP2A activity and DEX-induced glucocorticoid receptor nuclear translocation	PP2A	[14]
	up	miR-21	Regulates immune polarization by targeting IL12p35	IL12p35	[16]
	down	miR-125b	Regulates goblet cell differentiation	SPDEF	[9]
IPF	down	miR-let-7d	Inhibits TGF-β	HMGA2	[18]
	down	miR-26a	Promotes proliferation and differentiation of fibroblasts into myofibroblasts	HMGA2	[19, 20]
	down	miR-29	Antifibrotic	ECM related proteins	[21, 24, 25]
	down	miR-708-3p	Regulates ADAM17 (through GATA/STAT3 signaling pathway)	ADAM17	[26]
COPD	up	miR-195	Regulates Akt signaling	PHLPP2	[32]
	down	miR-146a/b	Negatively correlated with TNFα, IL-6, -8, LTB-4 and LTE-4	Unknown	[30]
	down	miR-218-5p	Negatively correlated with number of neutrophils, dendritic cells and T cells	Unknown	[31]
ALI	up	miR-155	Along with TREM-1, regulate levels of neutrophils, IL-6, IL-1β and TNFα	SOCS-1	[40]
	up	miR-429	Regulates inflammation (IL-1β, IL-6 and TNF-α) by targeting DUSP1	DUSP1	[41]
	up	miR-34b-5p	Regulates inflammation (IL-1β, IL-6 and TNF-α) and apoptosis	PGRN	[42]
	up	miR-181a	Apoptotic	BCL-2	[43]
	down	miR-17	Negatively regulates FoxA1 and other genes involved in apoptosis	FoxA1	[39]
	down	miR-125b	Regulates body weight, lung inflammation and survival of mice	TNFα transcripts	[36, 37]
	down	miR-454	Regulates permeability index and production of CXCL1, CXCL2, IL-6 and TNFα	CXCL12	[38]

Disease	Expression in disease	miRNA	Function	Target	Reference
BPD	down	miR-17˜92 cluster	Could be a biomarker to predict BPD development and possibly a target to prevent the disease	Unknown	[45]
	down	miR-206	Contribute for the progression of BPD by targeting FN1, which can drive extracellular matrix remodelling	FN1	[47]
	down	miR-876-3p	Could be involved on the alveolar destruction *in vivo*	Unknown	[48]
VIRAL INFECTIONS					
RV	down	miR-23b	Inhibits infection by RV1B by downregulating VLDL receptor	VLDL R	[51]
	down	miR-128, -155	Target RV1B and inhibit viral replication	RV1B	[52]
RSV	up	miR-let-7b, -let-7i, -30b, -221	Involved with regulators of the innate immune response such as NF-kβ and IFN type I	Unknown	[54]
	down	miR-146-5p, miR-let-7c-5p, -221, -345-5p	Altered in Hep-2 cells that are persistently infected with RSV (functional roles in RSV infection remain to be investigated)	Unknown	[56]
IV	up	miR-4776	Controls IV production through NFKBIB expression and modulation of NF-kβ production	NFKBIB	[59]
	down	miR-302a	Regulates IRF5 expression, afecting the production of TNFα, IFNβ, CCL2, CCL5, IL-6 and IL-8	IRF5	[58]
BACTERIAL INFECTIONS					
	up	miR-328	Regulates NTHi clearance	Unknown	[62]
	up	miR-23a-5p	Involved in *Mycobacterium tuberculosis* survival and induction of autophagy in infected macrophages	Unknown	[63]
	up	miR-155	Regulates IL-17 and IL-23 production and bacterial clearance in the lungs	Unknown	[64]