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The roles of miRNAs as potential biomarkers in lung diseases

Shamila D. Alipoor [a,b], Ian M. Adcock [c], Johan Garssen [d,e], Esmaeil Mortaz [a,c,f,*], Mohammad Varahram [g], Mehdi Mirsaeidi [h], Aliakbar Velayati [g]

[a] Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran
[b] Institute of Medical Biotechnology, Molecular Medicine Department, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran
[c] Cell and Molecular Biology Group, Airways Disease Section, National Heart and Lung Institute, Imperial College London, Dovehouse Street, London, UK
[d] Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands
[e] Nutricia Research Centre for Specialized Nutrition, Utrecht, The Netherlands
[f] Clinical Tuberculosis and Epidemiology Research Center, National Research and Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran
[g] Mycobacteriology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[h] Division of Pulmonary and Critical Care, University of Miami, Miami, FL, USA

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ABSTRACT

MicroRNAs (miRNAs) are small non-coding RNAs which can act as master regulators of gene expression, modulate almost all biological process and are essential for maintaining cellular homeostasis. Dysregulation of miRNA expression has been associated with aberrant gene expression and may lead to pathological conditions. Evidence suggests that miRNA expression profiles are altered between health and disease and as such may be considered as biomarkers of disease. Evidence is increasing that miRNAs are particularly important in lung homeostasis and development and have been demonstrated to be the involved in many pulmonary diseases such as asthma, COPD, sarcoidosis, lung cancer and other smoking related diseases. Better understanding of the function of miRNA and the mechanisms underlying their action in the lung, would help to improve current diagnosis and therapeutics strategies in pulmonary diseases. Recently, some miRNA-based drugs have been introduced as possible therapeutic agents. In this review we aim to summarize the recent findings regarding the role of miRNAs in the airways and lung and emphasise their potential therapeutic roles in pulmonary diseases.

Contents

* Corresponding author at: Department of Pharmaceutical Sciences, Utrecht University, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands. Tel.: +31(0) 30 253 7353.
E-mail address: E.Mortaz@imperial.ac.uk (E. Mortaz).

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1. Introduction

In recent years great efforts have been made towards understanding the molecular mechanisms underlying the occurrence and development of pulmonary diseases. In parallel with this; the discovery of microRNAs has opened a new window in the field of gene regulation. miRNAs are small, non-coding molecules which act as master regulators of cellular processes. They regulate gene translation by attenuating protein translation via promoting their mRNA degradation (Esquela-Kerscher and Slack, 2006; He and Hannon, 2004; Jones-Rhoades et al., 2006).

The first miRNA, named Lin-4, was identified in 1993 (Lee et al., 1993) and miRNAs have subsequently been shown to be present in a wide variety of species from single-cell algae to humans. Phylogenetically, this class of molecule emerged before the diversification of unicellular to multicellular organisms and may, therefore, be envisaged as a primal, critical and necessary regulatory mechanism present throughout evolution (Zhang and Lv, 2001; Cai et al., 2009).

The turning point in miRNA research was made when a signature of miRNA expression was discovered in cancer cells. Since that, significant advances have been made towards the understanding of the important role of miRNAs in cellular pathology and disease etiology (O'Connell et al., 2012). At the evolutionary level, the complexity of an organism correlates well with the number of miRNAs expressed. Mammals express the highest number of miRNAs (Zhang and Lv, 2001) with the human genome capable of expressing ~1000 miRNAs with tissue and cell specificity (O'Connell et al., 2012). miRNAs have the potential to target more than one hundred mRNAs and may therefore affect several biological pathways simultaneously (He and Hannon, 2004). Due to their importance in a wide array of biological processes, miRNAs have also been proposed as novel biomarkers for the diagnosis, treatment monitoring and prognosis of a broad range of diseases (George and Mittal, 2010; Guz et al., 2014; Zheng et al., 2011; Hennessey et al., 2012).

In the context of inflammatory responses, miRNAs have a central role in regulating the expression of key proteins that control the type and magnitude of the immune responses seen (O'Connell et al., 2012). MicroRNAs are important modifiers of the immune system and regulate human defense mechanisms. Recent research revealed central roles for miRNAs in different aspects of inflammation and disease pathogenesis. Here we aim to review recent advances in our understanding of the role of miRNAs in pulmonary diseases.

2. MiRNA properties, biogenesis and function

2.1. MiRNA genomics

Let7 and lin-4 were the first miRNAs found in 2001 (Lagos-Quintana et al., 2001) and there has been an explosion in the identification of miRNAs since this time. miRNAs are distributed across all human chromosomes except the Y chromosome. More than fifty percent of miRNA genes are located in clusters and produce polycistronic primary transcripts upon transcription (Kim and Nam, 2006). The expression of miRNAs in clusters may result from gene duplication and these cluster-associated miRNAs may target one or several genes in a specific pathway and suggests that they function as a co-ordinated unit (Bartel, 2004).

It was previously believed that miRNA genes were located in intergenic regions, but, it is now well accepted that many miRNA genome are located in defined transcription units (TUs) (Rodriguez et al., 2004). As such, protein coding genes may contain miRNAs located within their introns and exons as well as within the non-coding regions of the genome. Intronic miRNAs can be categorized as those located in either protein-coding or noncoding Tus and the position of some intronic miRNAs within the genome is conserved among diverse species. For example, in both insects and mammals, the location of miR-7 is found in the hnRNP K intron (Kim and Nam, 2006; Bartel, 2004).

2.2. miRNA Biogenesis

MicroRNA biogenesis is a two step process involving both a nuclear and cytoplasmic event. MicroRNA genes are transcribed in the nucleus by RNA polymerase II or III. The long primary transcript, known as pri-miR, has a large stem loop structure flanked by single-stranded RNA ends. Pri-miRNA processing involves nuclear cleavage of both ends by the "microprocessor" protein complex comprised of Drosha, an RNase type III endonulease, and several cofactors. These co-factors include the double-stranded RNA-binding protein DiGeorge syndrome Critical Region 8 (DGCR8). Pri-miRNA processing results in the generation of a short hairpin structure of 70–90 nucleotides named the precursor of mature miR (pre-miR). Pre-miRNAs are subsequently translocated from the nucleus into the cytoplasm via an active process involving exportin-5 (MacFarlane and Murphy, 2010) (Fig. 1).

In the cytoplasm, pre-miRNA is incorporated into a pre-miRNA processing complex composed of Dicer, the human immunodeficiency virus trans-activating response RNA binding protein (TRBP) and Ago2. This complex cleaves the pre-miRNA to leave a double stranded miRNA duplex of 19–25 nucleotides. One of these strands forms the mature miR strand (or guide strand) and the other forms a passenger strand (or miRNA* strand) with the miRNA* generally being degraded (Wahid et al., 2010). The single strand mature miR is subsequently incorporated into the RNA-induced silencing complex (RISC) (Fig. 2).

The loaded RISC complex enables miRNAs to recognize complementary sequences located in the 3′-untranslated regions (3′-UTR) of target mRNAs and promotes translational inhibition or degradation of mRNA (Fig. 3). Previous studies have shown that 3′ UTRs in mRNA target sites have an important role in miRNA: mRNA interactions. Genes with longer 3′-UTRs usually have a higher density of miRNA-binding sites whereas genes with shorter 3′-UTRs usually have a low density of miRNA-binding sites (MacFarlane and Murphy, 2010; Ha and Kim, 2014).

2.3. miRNA function and mechanism

The function of miRNAs in gene regulatory pathways is a key step in many biological processes. Each individual miRNA may be involved in the regulation of more than one mRNA and each mRNA in turn may be regulated by multiple miRNAs (Valinezhad Orang et al., 2014; Vidigal and Ventura, 2015). MicroRNAs affect gene expression via multiple mechanisms. Down regulation of gene expression by miRNAs can occur at three stages in the transcription/translation process; pretranslational, posttranslational or co-transcriptional silencing. In the pretranslation step, gene silencing

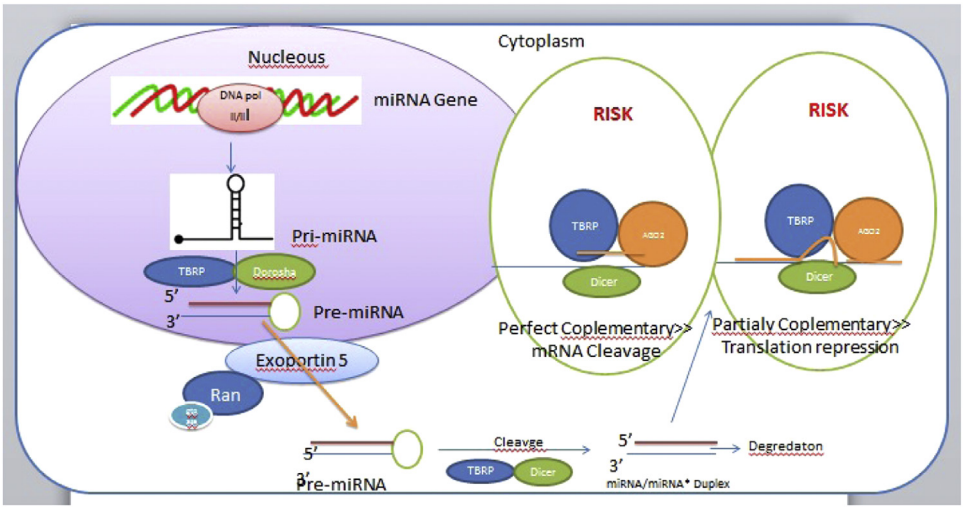

Fig. 1. mRNA biogenesis. microRNA (miRNA) genes are transcribed by Pol II and primary miRNA (pri-miRNAs) is produced. The next step is mediated by the microprocessor complex (which comprise of Drosha and DiGeorge syndrome critical region gene 8 (DGCR8)) that generates a stem-loop pre-miRNAs with 65 nucleotide. Pre-miRNA has 2-nt 3′ overhang, and is transported to cytoplasm by the nuclear export factor exportin 5 (EXP5). In the cytoplasm, RNase III Dicer produce miRNA duplexes. Dicer, TRBP and Argonaute (AGO) continue the processing of miRNA duplexes and the assembly of the RISC (RNA-induced silencing complex). One of the strands in duplex miRNA forms the mature miR strand that remains on the Ago protein and the other strand is degraded. The loaded RISC machinery guides the miRNA to recognize the target sequences on the mRNA target 3′-UTR. The degree of complementarity between these complementary sites determine the outcome of this interaction; which will give rise to degradation of the mRNA in perfect complementary condition or translation repression when the complementarily is lower.

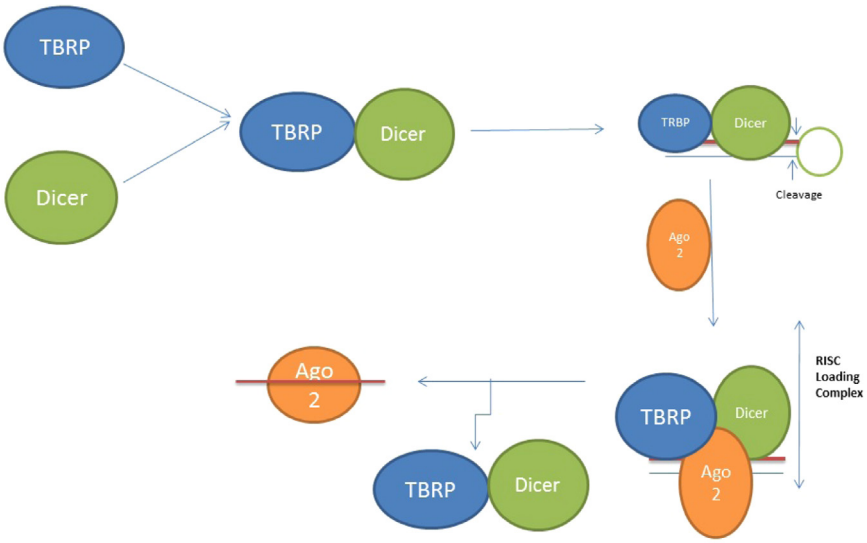

Fig. 2. RISC assembly and miRNA maturation. Dicer and TRBP firstly interact and recruit pre-miRNA after its export from nucleus. Then, Dicer catalyzes the production of the mature miRNA duplex. TRBP, Dicer and Ago2 form a tertiary complex and mediate RISC assembly and pre- miRNA processing. In continue, one of the strands remains on the Ago protein and forms mature miR whereas the other one is degraded.

occurs via a specialized RNA-induced transcriptional silencing (RITS) complex, containing nuclear Argonaute (Ago) protein, which probably acts through chromatin remodelling (Grewal and Elgin, 2007). MicroRNAs may also exert their effects on gene silencing via mRNA degradation following either deadenylation from the 3′ end or decapping from the 5′ end. Following the loss of the poly(A) tail and cap structure, the remaining mRNA is degraded by cytoplasmic exonucleases. mRNA cleavage may also occur by polysomal ribonuclease1 (PMR1) in a sequence-specific endonucleolytic manner (Valinezhad Orang et al., 2014; Vidigal and Ventura, 2015; Fabian et al., 2010; Garneau et al., 2007).

Argonaut can also attenuate the action of translation initiation factors (Iwasaki and Tomari, 2009). elF4E is an eukaryotic translation initiation factor and directs the ribosomes to the cap structure of mRNAs (Mathonnet et al., 2007). Argonate competes with elF4E for binding to the cap structure and can also interfere with the formation of the closed-loop mRNA structure which occurs upon mRNA circularization and which is essential for translation initiation (Eulalio et al., 2008).

miRNAs can also modulate gene expression at the post initiation step via several mechanisms. For example, translation initiation can occur by miRNP-AGO2 attaching to elF6 and preventing the association of the large ribosomal subunit to miRNA-targeted mRNA (Wang et al., 2008; Chendrimada et al., 2007). Another miRNA-mediated post initiation repression mechanism involves ribosomal subunit dissociation and premature termination; which occurs following the interaction of interfering miRNPs with translational elongation factors (Mathonnet et al., 2007; Chendrimada et al., 2007; Ding and Großhans, 2009).

The main mechanism involved in the post transcriptional gene regulation step is via miRNA-mRNA hybridization. The 6 to 8-nucleotides in the 5′ region of miRNA, known as the “seed”

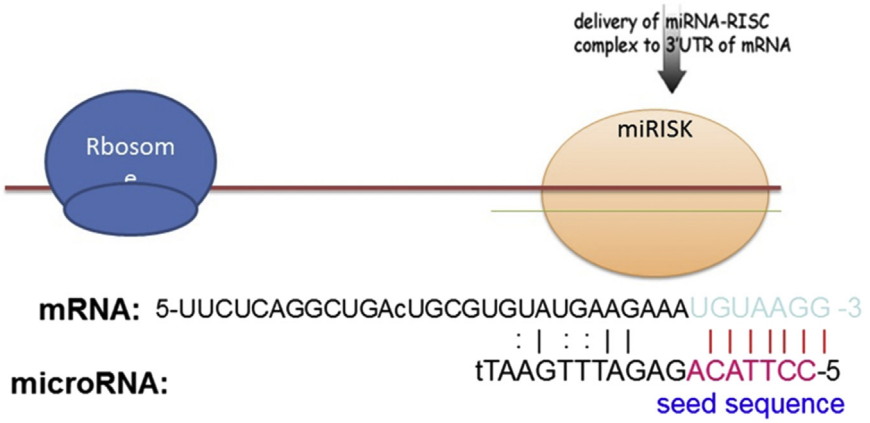

Fig. 3. MicroRNA-mRNA complex formation. Schematic overview of an interaction of miRNA and its target mRNA with Watson-Crick paring. The 6 to 8-nucleotides in the 5′region of miRNA, known as "seed" sequence is important in the interaction with target mRNA.

sequence, is responsible for the specificity of binding to mRNA targets. This region is highly conserved among species and any change in this sequence may affect its target spectrum (Cai et al., 2009). miRNAs interact with mRNA using Watson-Crick pairing which may be affected by several factors including the degree of complementarity between these paired sites. In addition, the accessibility of the paired sites, RNA secondary structures and the flanking sequence of the miRNA target site also influence the outcomes of hybridization (Valinezhad Orang et al., 2014). Overall, the degree of complementarity between these sites explains silencing: perfectly complementary give rise to degradation of the mRNA whilst translational repression occurs when the complementarily levels are lower (Bartel, 2004).

Recently cytoplasmic bodies mostly named as p body or GW bodies have been proposed to be involved in RNA degradation. These cytoplasmic processing bodies contain mRNA degradation-associated proteins such as hDcp1 and hLSm4 and the association of target mRNAs with these p body components results in their degradation (Sen and Blau, 2005; Eystathioy et al., 2003). In addition, in co-transcriptional gene silencing; miRNPs recruit protein decay factors which compete with elongation factors leading to degradation of the nascent protein (Pillai et al., 2007).

Although, the main mechanism of miRNA action in controlling gene expression is silencing or gene down regulation, in stress conditions such as hypoxia and nutrient shortage, some miRNAs appear to up-regulate selective mRNA targets. It is not clear whether this miRNA-mediated gene up-regulation is a global mechanism or is restricted to specific conditions (Cai et al., 2009; Valinezhad Orang et al., 2014; Vasudevan et al., 2007).

2.4. MicroRNAs and their potential pathogenicity

Given their impact on gene expression levels, miRNAs have a central regulatory role in different biological processes including development and cell signalling, proliferation, differentiation and apoptosis. MicroRNAs bind to complementary sequences on target mRNAs, leading to down regulation of gene expression via either translational repression or target degradation of the specific mRNA (Jonas and Izaurralde, 2015; Dong et al., 2013; van Rooij, 2011). Generally, miRNAs do not act as on-off switches but they fine-tune expression levels of central regulatory proteins to impact upon cellular phenotypes (Sevignani et al., 2006). Each miRNA is able to target up to hundreds of mRNAs in parallel and as such any change in the level of miRNA expression could result in a significant effect on many biological process and result in pathological states (van Rooij, 2011; Garzon et al., 2009; Winter et al., 2009).

In addition, sequence and length variation in miRNAs (isomiRs) may affect the capacity and specificity of miRNA targeting. These variations may be the result of cleavage steps performed by Drosha and Dicer enzymes or of genetic variants in the miRNA genome (Ryan et al., 2010). Variability may result in either the loss of regulation of target genes or an acquired down regulation of the gene targeted by the native miRNA. For example, a form of hereditary hearing loss is caused by a mutation in the miR-96 seed sequence (Mencía et al., 2009). MiR-96 is a member of miR-183 family which are expressed in sensory hair cells (Sacheli et al., 2009). Similarly, a heterozygous C-to-T transition within the seed region of miRNA-184 alters the stem-loop and secondary structure and is responsible for familial keratoconus in the eye (Hughes et al., 2011).

Duplication, deletion, or inversion of miRNAs genes could also cause pathologic conditions. A defect in the miRNA-17-92 polycistronic miRNA cluster encoding region results in microcephaly, short stature and digital abnormalities (de Pontual et al., 2011). Mutation or genetic variability may also occur in miRNA processing enzymes such as Drosha, Dicer, DGCR8, TRBP, Exportin-5 or AGO2. These mutations can lead to an insufficiency in the miRNA maturation process such as seen in DiGeorge syndrome where microdeletions in the DGCR8 gene at the 22q11.2 locus occurs (Zhang et al., 2015).

Alternatively, the microRNA genes themselves might be normal but their expression levels could change significantly leading to disease and/or an altered disease status. There are many reports highlighting the relationship between miRNA expression changes and many type of cancers including lung, breast and prostate (Garzon et al., 2009) as well as hereditary syndromes such as Down's Syndrome or Duchenne muscular dystrophy (Kawahara, 2014).

Identification of the key miRNAs that drive the pathologic condition is essential in order to define novel approaches. There are several databases available (e.g. miR2Disease (Jiang et al., 2009)) that show the association between specific miRNAs and human diseases (Li and Kowdley, 2012) and the increasing use of single cell RNA-seq analysis of disease and healthy subjects will rapidly expand our disease knowledge base. There is a huge amount of interest in the use of blood-based exosomal miRNAs as novel non-invasive biomarkers of disease severity of progression.

2.5. MicroRNAs in lung diseases

The function of miRNAs in lung development and their role in many pulmonary diseases has been studied. The lung has a unique and conserved miRNA expression profile (Sessa and Hata, 2013).

Almost all biological process including development and hemostasis, viral infection, inflammation and pulmonary disease are regulated by miRNAs. However, most of our knowledge regarding the role of miRNAs in lung pathology and development is mainly from animal models studies but the degree of translation into human disease is unclear (Sessa and Hata, 2013; Angulo et al., 2012).

The function of miRNAs in lung can be grouped into three categories. The first group are the miRNAs which are important in lung development, homeostasis and physiological functions. Here the level of miRNA expression varies during the different stages of lung maturation from the embryonic stage to the final stage of lung development (Sessa and Hata, 2013). Several miRNAs such as miR-155, miR-26a, let-7, miR-29, miR-15/miR-16, miR-223, miR-146a/b are classed within this group (Tomankova et al., 2010). miR-155 is important lung immunity since miR-155 deficient mice are immune deficient and fail to mount an efficient immunological response to exogenous stimuli (Rodriguez et al., 2007). miR-200c and miR-195 are uniquely only expressed in lung; however, nine other miRNAs are also expressed in other organs especially the heart (Wang et al., 2007).

miR-26a is the member of the lung miRNA family that are expressed in murine bronchial and alveolar epithelial cells. miR-26a targets the transcription factor SMAD-1 which is involved in the lung development process. The miR17-29 cluster is most highly expressed in early lung embryogenesis and significantly decreases throughout development (Mendell, 2008; Lu et al., 2007; Thomson et al., 2004). Overexpression of this miRNA cluster leads to an undifferentiated phenotype of lung epithelial progenitor cells in the mouse resulting from dysregulated cell proliferation (Lu et al., 2007).

Significant over expression of miR17-92 is reported in lung cancer (Hayashita et al., 2005) whilst silencing of the miR-17-92 cluster leads to enhanced expression of the pro-apoptotic protein Bim and inhibited B-cell development and these animals died shortly after birth (Tomankova et al., 2010). MicroRNA molecules which contribute in lung development are summarized in Table 1.

The second group of miRNAs are those involved in lung inflammation and regulation. This group includes miR-146a and miR-146b which play a central role in IL-1β activity at the onset of inflammation. Overexpression of these miRNAs causes down regulation of TNF-α and of other proinflammatory cytokines (Abd-El-Fattah et al., 2013).

MicroRNAs play a role in viral infection and are important in viral transmission at the initiation of infection. For example, miR200a and miR223 have a role in lethal influenza virus infection (Abd-El-Fattah et al., 2013). Up regulation of the miR-17 family, miR-574-5p and miR-214 have been observed at the onset of severe acute respiratory syndrome (SARS) infection (Hemida et al., 2010; Mallick et al., 2009). These miRNAs in combination with miR-223 and miR-98 target all four viral virulence proteins (Mallick et al., 2009). Some of the miRNAs in this group (Table 1) also regulate the inflammatory response to bacterial LPS.

The third group of miRNAs are directly involved in key lung functions associated with pulmonary disease pathophysiology and are discussed in detail below.

2.6. Smoking

The risk of succumbing to several lung diseases, such as chronic obstructive pulmonary disease (COPD) and lung cancer, is strongly associated with cigarette smoking and the respiratory epithelium of both these diseases express altered miRNA profiles. Comprehensive analysis of miRNA profiles in the bronchial epithelium of smokers shows that the expression of at least 28 miRNAs such as mIR-34c, let-7 family, miR-199, miR-218, and miR-222 are reduced in smokers. A reduction in the expression of these miRNAs promotes angiogenesis and potentially tumorigenicity (Angulo et al., 2012). miRNAs probably also directly influence the risk of developing tobacco addiction since mIR-504 expression induction seen with tobacco exposure increases the expression of dopamine receptor DRD1 gene expression (Huang and Li, 2009).

Wang et al. examined the profile of miRNA expression in the airway epithelium of subjects who quit smoking compared with those who never smoked. Even after 3 months of smoking cessation, dysregulated miRNAs levels were still reported. The target genes for these miRNAs were mostly involved in the Wnt/β-catenin signalling pathway (Wang et al., 2015a). This may explain why the risk of smoking related diseases such as COPD and cancer is significantly higher in compare to healthy non-smoker person even after quitting smoking, (Sessa and Hata, 2013; Angulo et al., 2012; Tomankova et al., 2010).

Table 1
Summary of miRNAs involved in the pathogenesis of pulmonary diseases.

	Up-regulated	References	Down-regulated	References
COPD	miR-223	(Ezzie et al., 2012)	miR-923	(Hassan et al., 2012)
	miR-1274	(Ezzie et al., 2012)	miR-937	(Hassan et al., 2012)
	miR-101	(Hassan et al., 2012)	miR-125b-1	(Hassan et al., 2012)
	miR-144	(Hassan et al., 2012)	miR-452	(Graff et al., 2012b)
	miR-146a	(Sato et al., 2010)	miR-452	(Graff et al., 2012b)
Asthma	miR-126	(Sato et al., 2010; Mattes et al., 2009)	Let-7	(Lu et al., 2011)
	miR-145	(Collison et al., 2011b)	miR-20b	(Song et al., 2012)
	miR-146a	(Collison et al., 2011b)	miR-133a	(Chiba et al., 2009)
	miR-146b	(Schembri et al., 2009)		
	miR-181	(Schembri et al., 2009)		
	miR-21	(Schembri et al., 2009)		
	miR-221	(Mayoral et al., 2011)		
	miR-222	(Mayoral et al., 2011)		
	miR-106a	(Sharma et al., 2012)		
	miR-156	(Bhattacharyya et al., 2011)		
Lung Cancer	miR-21	(Babu et al., 2011)	miR-106	(Karube et al., 2005; Chiba et al., 2009; Lin et al., 2010; Liu et al., 2011)
	miR-155	(Andrade et al., 2006)	miR-34	(Barger and Nana-Sinkam, 2015)
	miR-17-92	(Angulo et al., 2012)	miR-200	(Ahmad et al., 2014)
	miR-221/22	(Bhutta et al., 2014)	Let-7	(Bhaskaran et al., 2014)
	miR-205	(Balakrishnan et al., 2009)	miR-548	(Hu et al., 2014)
			miR-29	(Fabbri et al., 2007)
			miR-15a/16 cluster	(Bandi et al., 2009; Bandi and Vassella, 2011)

Let-7, a tumour suppressor miRNA, is down regulated by smoking (Angulo et al., 2012) and, conversely, cyclin F which is directly targeted by let-7 is significantly up-regulated by cigarette smoke (CS) exposure (Momi et al., 2014). CS exposure may cause mutations within miRNA genes to modify their expression or function. The presence of mutations within seed sequences or miRNA target sites may affect the susceptibility towards CS (Momi et al., 2014) and suggests that miRNAs might play a pivotal role in the pathogenesis of smoking-related diseases (Table 1).

2.7. Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the world and it is expected to become the third leading cause by 2020 (Angulo et al., 2012). COPD is defined as slowly progressive airflow obstruction associated abnormalities in the small airways. This is associated enhanced infiltration of immune and inflammatory cells, thickening of the alveolar wall and ultimately damage of the lung parenchymal and epithelium (Shi et al., 2009). COPD is, therefore, a mixture of emphysema, small airways inflammation and bronchitis (Hogg et al., 2004).

Studying the aberrant miRNA profiles seen in COPD patients compared with healthy controls has indicated the key role of the transforming growth factor (TGF)-β, Wnt and focal adhesion pathways in the pathogenesis of COPD (Angulo et al., 2012). A reduction in mIR-146a and let-7c expression levels in COPD has been implicated in the inflammatory state and the progression of COPD (Angulo et al., 2012). Smoking is the main risk factor of COPD pathogenicity (Ezzie et al., 2012). In COPD subjects who smoked, abnormal expression of seventy miRNAs was observed in comparison to healthy smokers (Sessa and Hata, 2013). The expression of mIR-101 and mIR-144 is increased in COPD patients and can be induced by exposure of human airway epithelial cells to tobacco smoke extract. These miRNAs suppress the cystic fibrosis transmembrane conductance regulator (CFTR) protein (which acts as a chloride channel) (Sessa and Hata, 2013) and may link smoking to subjects with bronchiectasis and CFTR-associated disorders.

MicroRNA profiling studies in human alveolar macrophages from smokers and non-smokers demonstrate general down regulation of miRNA clusters. The expression of miR-452 is reduced in smokers (Graff et al., 2012a). This miRNA targets matrix metalloproteinase-12 (MMP-12) which is associated with the development of emphysema (O'Leary et al., 2014). Altered macrophage miRNA expression was linked to the switch from an M2 to M1 polarization of alveolar macrophages in COPD patients (Graff et al., 2012a).

MicroR-146a is down-regulated by inflammatory pathways in vitro and in vivo in smokers and in COPD patients (Angulo et al., 2012; Wang et al., 2015b; Sato et al., 2010; Cornett and Lutz, 2014). Reduction of miR-146a results in the enhanced expression of COX2 (Cornett and Lutz, 2014). COX2 is an enzyme which is involved in the production of prostaglandin E2 (PGE2) (Cornett and Lutz, 2014). The reduction in miR-146a expression in COPD lung tissue is accompanied by enhanced PGE2 (Sato et al., 2010). PGE2 is an important mediator of tissue inflammation in airway epithelium inducing growth of fibroblasts and collagen synthesis. PGE2 overproduction, associated with reduced miR-146a expression causes a reduction in the repair capacity of the lung (Sato et al., 2010), and is correlated with the severity of COPD (Angulo et al., 2012).

The expression of let-7c is diminished in COPD patients particularly in those who are active smokers. Let-7c targets tumour necrosis factor receptor type II (TNFR2) which has been implicated in COPD pathogenesis. There is also a correlation between the level of let-7c and forced expiratory volume-1 (FEV1) (Van Pottelberge et al., 2011). The levels of mir15/107 family members, which modulate TGF-β and Wnt pathways which are key signalling pathways in many diseases are elevated in COPD (Swallow et al., 2007).

Peripheral muscle dysfunction is a limiting factor in many COPD patients and is associated with high mortality and poor quality of life (Maltais et al., 2000; Gea et al., 2013). MicroR-1 is down regulated in COPD patients and a correlation between miR-1 expression and FEV1 and percentage of slow muscle fibres has been demonstrated (Angulo et al., 2012). Decreased miR-1 expression is also associated with greater levels of histone deacetylase 4 protein in muscular cells which can result in fibre type change and muscle weakness (Lewis et al., 2012).

2.8. Asthma

Asthma is a heterogeneous chronic inflammatory disease characterized by airways obstruction and hyper-responsiveness. Asthma is commonly associated with an abnormal response of the Th2-type CD4+T lymphocytes against certain antigens that followed with up-regulation of the related cytokines such as interleukin IL-4, IL-5 and IL-13 and increased levels of circulating IgE (Locksley, 2010; Kumar and Ghosh, 2009). Consequently, IgE leads to release of chemical mediators such as histamine and leukotrienes that induce smooth muscle contraction and airway edema upon cross-linking of IgE molecules on IgE receptors on basophils and mast cells (Kumar and Ghosh, 2009; Wenzel, 2012).

miR-21 is over expressed in airway epithelial cells of patients with asthma (Wu et al., 2014) and may have a proinflammatory role by negatively regulating IL-12 expression. IL-12 helps to maintain the Th1/Th2 balance. Therefore, decreased IL-12 expression in asthmatic patients may be followed by an excessive Th2 response, or conversely, increased IL-12 expression may be accompanied with an enhanced Th1 response (Wu et al., 2014; Lu et al., 2009; Sawant et al., 2015).

Another miRNA which found to be overexpressed in lung of asthmatic patient was miR-126 (Perry et al., 2015). The inhibition of miR-126 suppresses the Th2 lymphocytes activation and prevents airway hypersensitivity. MicroR-21 and miR-126 expression is correlated with IL-13 concentration which is a major inflammatory factor in induction of an asthmatic attack (Wu et al., 2014). Animal models of asthma also demonstrate over expression of miR-21 and miR-26 in airway epithelial cells (Wu et al., 2014; Collison et al., 2011a; Lu et al., 2011) and this is linked to response to therapeutic intervention (Wu et al., 2014). Chiba et al. observed that a reduction of mir-133a was followed by increased expression of RhoA and increase bronchial hyperactivity in a murine model of asthma (Chiba and Misawa, 2010). In other studies, miR-1 and miR-145 inhibited lung inflammation in a mouse asthma model (Perry et al., 2015).

2.9. Sarcoidosis

Sarcoidosis is a granulomatous disease with unknown etiology that mostly involves the lung. Sarcoidosis can be defined as an inflammatory disease with enhanced immunologic hypersensitivity to unknown tissue antigens (Abd-El-Fattah et al., 2013; Jazwa et al., 2015). Previous studies reveal a powerful link between host genetics and the manifestations of the disease, however, it was observed that DNA polymorphisms are not enough to fully explain the phenotypic variability (Crouser et al., 2012). Therefore, there is a huge interest in identifying biomarkers for the detection and better understanding the mechanism(s) underlying the development of this disease and miRNAs have been proposed as critical disease regulators as well as being possible disease

biomarkers (Jazwa et al., 2015). A number of microarray analysis have been performed studying the pattern of miRNA expression with a view to understanding their possible roles in the pathogenesis of sarcoidosis. These studies showed a distinct population of miRNAs with a different pattern of expression in the lung and PBMC of sarcoidosis patients. These groups of miRNAs were predicted to target TGFβ and related WNT pathways which are probably important in disease pathology (Crouser et al., 2012). The abnormal patterns of tissue miRNA expression was shown to be strongly associated with pathological conditions and could promote fibrotic and obstructive lung disease (Maertzdorf et al., 2012).

PBMCs isolated from sarcoidosis patients express raised levels of mIR-34a compared to that seen in PBMCs from healthy controls (Jazwa et al., 2015). mIR-34a may down regulate sirtuin (SIRT)1 expression and stimulate the IFN-γ expression in sarcoidosis. SIRT1 is a major regulator of energy metabolism and tissue survival, therefore, it's inhibition leads to disruption of cell energy metabolism and induction of inflammatory responses mediated by NF-κB activation (Jazwa et al., 2015). The role of altered tissue miRNA expression in the pathogenesis of sarcoidosis have not yet been defined and requires more studies (Jazwa et al., 2015).

2.10. Lung Cancer

Lung cancer (LC) with an incidence of over 200,000 new cases per year is the leading cause of cancer-related deaths worldwide (Tarver, 2012). LC is classified into two main subtypes: small-cell (SCLC) and non-small-cell lung carcinoma (NSCLC). SCLC is more aggressive than NSCLC, frequently metastasizes and covers 12% of all cases (Guz et al., 2014). miRNAs have been implicated in the regulation of all the cellular pathways including differentiation, proliferation and survival linked to cancer and so abnormalities in the expression of miRNAs may be expected to play a role in different types of cancer including lung cancer (Angulo et al., 2012). Indeed, a reduction in the levels of Dicer which is necessary for miRNA maturation has been reported in lung cancer (Karube et al., 2005).

A number of different genetic alterations underpinning the genesis of cancer can modulate miRNA expression and maturation (Cherni and Weiss, 2011). miR-let 7 was the first miRNA whose expression was reported as abnormal in lung cancer and reduced post-operative survival is correlated with attenuated let-7 expression (Leidinger et al., 2011). HRAS, KRAS, and NRAS which are the members of the RAS GTPase family, contain multiple complementary binding sites for let-7 in their 3′-UTR and so let-7 is considered as a negative regulator of the RAS oncogene family (Guz et al., 2014). Let-7 can also modulate the expression of other proto-oncogenes involved in the G1/S transition such as CDC 25a, CDK16, and cyclin D that are (Leidinger et al., 2011). In addition, it has been recently reported that let-7 targets BCL-2, a proto-oncogene involved in the regulation of apoptosis (Esquela-Kerscher et al., 2008). In vivo experiments confirmed the important role of let-7 in growth suppression and the induction of apoptosis (Esquela-Kerscher et al., 2008).

MicroR-21 is another miRNA that is overexpressed in NSCLC patients and its expression negatively correlated with overall survival in NSCLC patients (Markou et al., 2008). Overexpression of miR-21 was demonstrated in both smokers and non-smokers with lung cancer (Seike et al., 2009). miR-21 regulates tumour suppressor genes including programmed cell death 4 (PDCD4) and phosphatase and tensin homologue (PTEN) (Zhang et al., 2010). In some lung cancer subjects, mostly never-smokers, the epidermal growth factor receptor (EGFR) carries a mutation that leads to constitutive activation of tyrosine kinase (TK) and tumour progression (Leidinger et al., 2011) and miR-21 is significantly up-regulated in lung cancer subjects with EGFR mutations (Seike et al., 2009). mIR-21 expression is positively regulated by the EGFR signalling pathway in lung cancer (Seike et al., 2009). Dysregulation of mIR-21 is also reported in several other types of cancers and is thought to be a general oncomir without tissue specificity (Leidinger et al., 2011).

Overexpression of members of the mir17-92 cluster was observed in NSCLC patients (Hayashita et al., 2005; Calin and Croce, 2006) and mIR-31, which promotes cell growth and represses apoptosis and cell death, is increased in NSCLC. Elevated levels of these miRNAs results in the reduced expression of tumour suppressor genes land increased tumour growth and metastasis (Liu et al., 2010).

In contrast, the expression of miR- 34 is reduced in lung tumours. Transcription of mIR-34 is directly induced by the tumour suppressor p53 in response to DNA damage and inhibits inappropriate cell proliferation (He et al., 2007). A reduction in the levels of miR-1 and miR-133b was also reported in A549 and adenocarcinoma (H2009) cell lines respectively (Crawford et al., 2009; Nasser et al., 2008). These miRNAs function by targeting pro-survival molecules MCL-1/BCL2L2 and oncogenic targets such as MET/Pim-1 respectively. The reduction of these miRNAs causes an increase in MET, Pim-1, HDAC4, MCL-1, BCL2L2 which are involved in pulmonary carcinogenesis (Crawford et al., 2009; Nasser et al., 2008). MicroR- 218 was also reported to be down regulated in the subjects with NSCLC (Davidson et al., 2010). There are numerous reports on other miRNAs that are dysregulated in lung cancer as shown in Table 1.

2.11. MicroRNAs as therapy and as diagnostic tools in lung disease

Despite of recent progress in the understanding the miRNA roles and their mechanism of function in biological pathways, there are still many obstacles to overcome prior to miRNAs technology entering the clinic. These obstacles include miRNA drug delivery, stability and tissue specificity of the therapeutic agent. It is critical to improve our understanding of drug pharmacokinetics as well as minimizing the off-target effects and toxicity (Jackson and Linsley, 2010). Currently two strategies are being used based on restoring or blocking miRNA function particularly targeting the activity and function of tumour suppressive miRNAs. For example, hypomethylating agents such as decitabine or 5′-azacytidine may be applied to reverse epigenetic silencing of miRNAs (Al-Ali et al., 2014). A more specific approach, however, is to use miRNA mimics. MicroRNA mimics are synthetic, chemically modified double stranded short RNAs that can mimic native miRNAs and can be designed to have exquisite selectivity based on a miRNA sequence. Some modifications in the miRNA mimic base sequence is required to enhance uptake or increase their stability to prevent RISC loading.

Nanoparticle and liposome have also been used to improve the uptake of miRNA mimics. For example, systematic delivery of let-7 and miR-34 mimics using a neutral lipid emulsion inhibits lung tumour growth in a KRAS model of murine lung cancer (Trang et al., 2011) and delivery of miR-7 to an EGFR-resistant model of lung cancer leads to a significantly reduction in tumour volume (Rai et al., 2011). Reducing off-target effects of miRNA therapy in lung cancer therapy can be achieved by coating nanoparticles with tumour-specific antibodies. In a model of metastatic melanoma, delivery of miR-34 by coated nanoparticles reduced lung metastasis (Chen et al., 2010). MicroR-34 loss-of-function mutation have been demonstrated in lung cancer (Shi et al., 2014; Xue et al., 2014) and animal models have shown that restoration of miR-34 could lead to regression of tumour growth (Wiggins et al., 2010; Craig et al., 2012; Bader, 2012). MRX34 is a miR-34 mimic encapsulated in a liposomal nanoparticle and is the first miRNA

mimic to enter clinical trials (Bouchie, 2013).

Vector based delivery systems have also been used in several studies. Lentiviral and adenoviral delivery of let-7, for example, caused a significant reduction of tumour growth in a mouse model of lung cancer with a KRAS mutation (Esquela-Kerscher et al., 2008; Trang et al., 2010).

Anti-sense oligonucleotide-based approaches have also been used to block onco-miRNAs. These methods include antagomirs, locked nucleic acid (LNA) miRNAs and miRNA sponges. Antagomirs also known as anti-miRs or blockmirs are engineered synthetic oligonucleotides that bind specifically to particular microRNAs and disrupt their function (Scherr et al., 2007). Similar to antagomirs, sponge RNAs are small synthetic RNAs containing multiple tandem binding sites complementary to a heptamer in the seed sequence of the targeted miRNA which allow the sponge to block an entire miRNA seed family (Ebert and Sharp, 2010). A locked nucleic acid (LNA) is a modified RNA molecule in which the ribose ring in the nucleic acid analogue is "locked" by a methylene bridge connecting the 2′-O and the 4′-C groups. This class of LNA antisense drugs have been improved in comparison with previous generations of antisense drugs and have been widely used in in vitro and in vivo experiments to inhibit targeted miRNAs. "Miravirsen" is a LNA drug against miR-122, recently entered clinical trials (Ørum, 2014).

Recent advances have highlighted the potential of miRNAs in the diagnosis of lung cancer to complement lung low dose CT-scan (LDCT) screening order to reduce the false positive rate. Although LDCT is the current gold standard for early detection of lung cancer (Abd-El-Fattah et al., 2013) it produces over-diagnosis of indolent nodules. Most of these false positives could be successfully overcome by using a combination of miRNA detection assays as with imaging (Chakravarthy et al., 2007; Boeri et al., 2015).

3. Conclusion

MicroRNAs not only regulate cellular behaviour at baseline but also under various stress conditions and in disease. Indeed, miRNAs act as a web of mediators that modulate nearly all biological process. The lung is constantly exposed to various stresses such as chemical irritants, free radicals and air pollutants and it is likely that miRNAs play a crucial role in the host defense against these exogenous factors. Aberrant miRNA expression profiles have been reported in most lung diseases and it is likely that dysregulation of miRNAs is a major driver in the pathogenesis of many pulmonary diseases including cancer and other smoking related diseases. Better understanding of the underlying mechanisms of dysregulated miRNA expression and the relationship with their target genes would provide further insight for the use of microRNAs as prognostic or therapeutic biomarkers.

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