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The roles of miRNAs as potential biomarkers in lung diseases

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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.

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 [1-3].

The first miRNA, named Lin-4, was identified in 1993 [4] 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 [5, 6].

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 understanding the important role of miRNAs in cellular pathology and disease aetiology [7]. At the evolutionary level, the complexity of an organism correlates well with the number of miRNAs expressed. Mammals express the highest number of miRNAs [5] with the human genome capable of expressing ~1,000 miRNAs with tissue and cell specificity [7]. miRNAs have the potential to target more than one hundred mRNAs and may therefore affect several biological pathways simultaneously [2]. 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 [8-11].

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 [7]. 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.

MiRNA Properties, Biogenesis and function

**miRNA genomics**

Let7 and lin-4 were the first miRNAs found in 2001 [12] 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 [13]. 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 [14].

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) [15]. 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 [13, 14].

**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 endonuclease, 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 [16].

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 [17]. 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 [16,18].

**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 [19, 20]. 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 cotranscriptional silencing. In the pretranslation step, gene silencing occurs via a specialized RNA-induced transcriptional silencing (RITS) complex, containing nuclear Argonaute (Ago) protein, which probably acts through chromatin remodelling [21]. 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 [19, 20, 22, 23].
Argonaut can also attenuate the action of translation initiation factors [24]. eIF4E is an eukaryotic translation initiation factor and directs the ribosomes to the cap structure of mRNAs [25]. Argonaute competes with eIF4E 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 [26].

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 eIF6 and preventing the association of the large ribosomal subunit to miRNA-targeted mRNA [27, 28]. 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 [28, 29, 25].

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” 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 [6]. 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 [19]. 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 [14].

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 [30, 31]. In addition, in co-transcriptional gene silencing; miRNPs recruit protein decay factors which compete with elongation factors leading to degradation of the nascent protein [32].

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 [6, 33, 19].

**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 [34-36]. Generally, miRNAs do not act as on-off switches but they fine-tune expression levels of central regulatory proteins to impact upon cellular phenotypes [37]. 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 [38, 36, 39].

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 [40]. 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 [41]. MiR-96 is a member of miR-183 family which are expressed in sensory hair cells [42]. 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 [43].

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 [44]. 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 [45]. 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 [38] as well as hereditary syndromes such as Down’s Syndrome or Duchenne muscular dystrophy [46].

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 [47]) that show the association between specific miRNAs and human diseases [48] 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.

**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 [49]. 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 [49, 50].

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 [49]. 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 [51]. 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 [52]. miR-200c and miR-195 are uniquely only expressed in lung; however, nine other miRNAs are also expressed in other organs especially the heart [53].

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 [54-56]. Overexpression of this miRNA cluster leads to an undifferentiated phenotype of lung epithelial progenitor cells in the mouse resulting from dysregulated cell proliferation [55]. Significant over expression of miR17-92 is reported in lung cancer [57] 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 [51]. 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 [58]. 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 [58]. 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 [59, 60]. These miRNAs in combination with miR-223 and miR-98 target all four viral virulence proteins [60]. 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.

**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 [50]. 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 [61].
Wang and colleagues 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 signaling pathway [62]. 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, [51, 49, 50].

Let-7, a tumour suppressor miRNA, is down regulated by smoking [50] and, conversely, cyclin F which is directly targeted by let-7 is significantly up-regulated by cigarette smoke (CS) exposure [63]. 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 [63] and suggests that miRNAs might play a pivotal role in the pathogenesis of smoking-related diseases (Table 1).

**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 [50]. 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 [64]. COPD is, therefore, a mixture of emphysema, small airways inflammation and bronchitis [65].

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 [50]. A reduction in miR-146a and let-7c expression levels in COPD has been implicated in the inflammatory state and the progression of COPD [50]. Smoking is the main risk factor of COPD pathogenicity [66]. In COPD subjects who smoked, abnormal expression of seventy miRNAs was observed in comparison to healthy smokers [49]. 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) [49] 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 [67]. This miRNA targets matrix metalloproteinase-12 (MMP-12) which is associated with the development of emphysema [68]. Altered macrophage miRNA expression was linked to the switch from an M2 to M1 polarization of alveolar macrophages in COPD patients [67].

MicroRNA-146a is down-regulated by inflammatory pathways in vitro and in vivo in smokers and in COPD patients [69-71, 50]. Reduction of miR-146a results in the enhanced expression of COX2 [71]. COX2 is an enzyme which is involved in the production of prostaglandin E2 (PGE2) [71]. The reduction in miR-146a expression in COPD lung tissue is accompanied by enhanced PGE2 [70]. 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 [70], and is correlated with the severity of COPD [50].

The expression of let-7c is diminished in COPD patients particularly in those who are active smokers. Let-7c targets tumor 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) [72]. 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 [73].

Peripheral muscle dysfunction is a limiting factor in many COPD patients and is associated with high mortality and poor quality of life [74, 75]. MicroRNA-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 [50]. 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 [76].

**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 [77, 78]. 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 [79, 78].

miR-21 is over expressed in airway epithelial cells of patients with asthma [80] 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 [81, 80, 82].

Another miRNA which found to be overexpressed in lung of asthmatic patient was miR-126 [83]. 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 [80]. Animal models of asthma also demonstrate over expression of miR-21 and miR-26 in airway epithelial cells [84, 85, 80] and this is linked to response to therapeutic intervention [80]. Chiba and colleagues observed that a reduction of mir-133a was followed by increased expression of RhoA and increase bronchial hyperactivity in a murine model of asthma [86]. In other studies, miR-1 and miR-145 inhibited lung inflammation in a mouse asthma model [83].

**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 [87, 58]. 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 [88]. 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 [87]. 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 [88]. The abnormal patterns of tissue miRNA expression was shown to be strongly associated with pathological conditions and could promote fibrotic and obstructive lung disease [89].

PBMCs isolated from sarcoidosis patients express raised levels of mIR-34a compared to that seen in PBMCs from healthy controls [87]. 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 [87]. The role of altered tissue miRNA expression in the pathogenesis of sarcoidosis have not yet been defined and requires more studies [87].

**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 [90]. 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 [9]. 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 [50]. Indeed, a reduction in the levels of Dicer which is necessary for miRNA maturation has been reported in lung cancer [94].

A number of different genetic alterations underpinning the genesis of cancer can modulate miRNA expression and maturation [91]. 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 [92]. 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 [9]. Let-7 can also modulate the
expression of other proto-oncogenes involved in the G1/S transition such as CDC25a, CDK16, and cyclin D that are [92]. In addition, it has been recently reported that let-7 targets BCL-2, a proto-oncogene involved in the regulation of apoptosis [93]. In vivo experiments confirmed the important role of let-7 in growth suppression and the induction of apoptosis [93].

MicroR-21 is another miRNA that is overexpressed in NSCLC patients and its expression negatively correlated with overall survival in NSCLC patients [101]. Overexpression of miR-21 was demonstrated in both smokers and non-smokers with lung cancer [102]. miR-21 regulates tumour suppressor genes including programmed cell death 4 (PDCD4) and phosphatase and tensin homologue (PTEN)[103]. 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 tumor progression [92] and miR-21 is significantly up-regulated in lung cancer subjects with EGFR mutations [102]. miR-21 expression is positively regulated by the EGFR signalling pathway in lung cancer [102]. Dysregulation of miR-21 is also reported in several other types of cancers and is thought to be a general oncomir without tissue specificity [92].

Overexpression of members of the mir17-92 cluster was observed in NSCLC patients [57, 95] 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 [96].

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 [97]. A reduction in the levels of miR-1 and miR-133b was also reported in A549 and adenocarcinoma (H2009) cell lines respectively [98, 99]. 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 [99, 98]. MicroR-218 was also reported to be down regulated in the subjects with NSCLC [100]. There are numerous reports on other miRNAs that are dysregulated in lung cancer as shown in Table 1.
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 [104]. 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 [105]. 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 [106] and delivery of miR-7 to an EGFR-resistant model of lung cancer leads to a significantly reduction in tumour volume [107]. 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 [108]. MicroR-34 loss-of-function mutation have been demonstrated in lung cancer [109, 110] and animal models have shown that restoration of miR-34 could lead to regression of tumour growth [111-113]. MRX34 is a miR-34 mimic encapsulated in a liposomal nanoparticle and is the first miRNA mimic to enter clinical trials [114].

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 tumor growth in a mouse model of lung cancer with a KRAS mutation [115, 93].

Anti-sense oligonucleotide-based approaches have also been used to block oncomiRNAs. 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 [116]. 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 [117]. 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 [118].

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 [58] 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 [119, 120].

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

|                  | Up-regulated | References | Down-regulated | References |
|------------------|--------------|------------|----------------|------------|
| **COPD**         | miR-223      | [66]       | miR-923        | [121]      |
|                  | miR-1274     | [66]       | miR-937        | [121]      |
|                  | miR-101      | [121]      | miR-125b-1     | [121]      |
|                  | miR-144      | [121]      | miR-452        | [122]      |
|                  | miR-146a     | [70]       | miR-452        | [122]      |
| **Asthma**       | miR-126      | [70, 123]  | Let-7          | [85]       |
|                  | miR-145      | [125]      | miR-20b        | [124]      |
|                  | miR-146a     | [125]      | miR-133a       | [126]      |
|                  | miR-146b     | [127]      |                |            |
|                  | miR-181      | [127]      |                |            |
|                  | miR-21       | [127]      |                |            |
|                  | miR-221      | [128]      |                |            |
|                  | miR-222      | [128]      |                |            |
|                  | miR-106a     | [129]      |                |            |
|                  | miR-156      | [130]      |                |            |
| **Lung Cancer**  | miR-21       | [133]      | miR-106        | [94, 126, 131, 132] |
|                  | miR-155      | [135]      | miR-34         | [134]      |
|                  | miR-17-92    | [50]       | miR-200        | [136]      |
|                  | miR-221/22   | [138]      | Let-7          | [137]      |
|                  | miR-205      | [140]      | miR-548        | [139]      |
|                  |              |            | miR-29         | [141]      |
|                  |              |            | miR-15a/16 cluster | [142, 143] |
Figure legends

Figure 1: 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.

Figure 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.

Figure 3: 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.
Reference List

1. Esquela-Kerscher A, Slack FJ. Oncomirs—microRNAs with a role in cancer. Nature Reviews Cancer. 2006;6(4):259-69.

2. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nature Reviews Genetics. 2004;5(7):522-31.

3. Jones-Rhoades MW, Bartel DP, Bartel B. MicroRNAs and their regulatory roles in plants. Annu Rev Plant Biol. 2006;57:19-53.

4. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993;75(5):843-54.

5. Zhang Y, Lv Q. Comparative Analysis of miRNA-mediated Gene Regulation in Mammals. eLS. 2001.

6. Cai Y, Yu X, Hu S, Yu J. A brief review on the mechanisms of miRNA regulation. Genomics, proteomics & bioinformatics. 2009;7(4):147-54.

7. O’Connell RM, Rao DS, Baltimore D. microRNA regulation of inflammatory responses. Annual review of immunology. 2012;30:295-312.

8. George GP, Mittal RD. MicroRNAs: Potential biomarkers in cancer. Indian journal of clinical biochemistry: IJCB. 2010;25(1):4-14.

9. Guz M, Rivero-Muller A, Okon E, Stenzel-Bembenek A, Polberg K, Slomka M et al. microRNAs-role in lung cancer. Disease markers. 2014;2014:218169.

10. Zheng D, Haddadin S, Wang Y, Gu LQ, Perry MC, Freter CE et al. Plasma microRNAs as novel biomarkers for early detection of lung cancer. International journal of clinical and experimental pathology. 2011;4(6):575-86.

11. Hennessey PT, Sanford T, Choudhary A, Mydlarz WW, Brown D, Adai AT et al. Serum microRNA biomarkers for detection of non-small cell lung cancer. PloS one. 2012;7(2):e32307.

12. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. Science. 2001;294(5543):853-8.

13. Kim VN, Nam J-W. Genomics of microRNA. TRENDS in Genetics. 2006;22(3):165-73.

14. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. cell. 2004;116(2):281-97.
15. Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A. Identification of mammalian microRNA host genes and transcription units. Genome research. 2004;14(10a):1902-10.

16. MacFarlane L-A, Murphy PR. MicroRNA: biogenesis, function and role in cancer. Current genomics. 2010;11(7):537.

17. Wahid F, Shehzad A, Khan T, Kim YY. MicroRNAs: synthesis, mechanism, function, and recent clinical trials. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2010;1803(11):1231-43.

18. Ha M, Kim VN. Regulation of microRNA biogenesis. Nature reviews Molecular cell biology. 2014;15(8):509-24.

19. Valinezhad Orang A, Safaralizadeh R, Kazemzadeh-Bavili M. Mechanisms of miRNA-Mediated Gene Regulation from Common Downregulation to mRNA-Specific Upregulation. International journal of genomics. 2014;2014:970607.

20. Vidigal JA, Ventura A. The biological functions of miRNAs: lessons from in vivo studies. Trends in cell biology. 2015;25(3):137-47.

21. Grewal SI, Elgin SC. Transcription and RNA interference in the formation of heterochromatin. Nature. 2007;447(7143):399-406.

22. Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by microRNAs. Annual review of biochemistry. 2010;79:351-79.

23. Garneau NL, Wilusz J, Wilusz CJ. The highways and byways of mRNA decay. Nature reviews Molecular cell biology. 2007;8(2):113-26.

24. Iwasaki S, Tomari Y. Argonaute-mediated translational repression (and activation). Fly. 2009;3(3):204-6.

25. Mathonnet G, Fabian MR, Svitkin YV, Parsyan A, Huck L, Murata T et al. MicroRNA inhibition of translation initiation in vitro by targeting the cap-binding complex eIF4F. Science. 2007;317(5845):1764-7.

26. Eulalio A, Huntzinger E, Izaurralde E. Getting to the root of miRNA-mediated gene silencing. Cell. 2008;132(1):9-14.

27. Wang B, Yanez A, Novina CD. MicroRNA-repressed mRNAs contain 40S but not 60S components. Proceedings of the National Academy of Sciences. 2008;105(14):5343-8.

28. Chendrimada TP, Finn KJ, Ji X, Baillat D, Gregory RI, Liebhaber SA et al. MicroRNA silencing through RISC recruitment of eIF6. Nature. 2007;447(7146):823-8.
29. Ding XC, Großhans H. Repression of C. elegans microRNA targets at the initiation level of translation requires GW182 proteins. The EMBO journal. 2009;28(3):213-22.

30. Sen GL, Blau HM. Argonaute 2/RISC resides in sites of mammalian mRNA decay known as cytoplasmic bodies. Nature cell biology. 2005;7(6):633-6.

31. Eystathioy T, Jakymiw A, CHAN EK, Séraphin B, Cougot N, Fritzler MJ. The GW182 protein colocalizes with mRNA degradation associated proteins hDcp1 and hLSm4 in cytoplasmic GW bodies. rna. 2003;9(10):1171-3.

32. Pillai RS, Bhattacharyya SN, Filipowicz W. Repression of protein synthesis by miRNAs: how many mechanisms? Trends in cell biology. 2007;17(3):118-26.

33. Vasudevan S, Tong Y, Steitz JA. Switching from repression to activation: microRNAs can up-regulate translation. Science. 2007;318(5858):1931-4. doi:10.1126/science.1149460.

34. Jonas S, Izaurrealde E. Towards a molecular understanding of microRNA-mediated gene silencing. Nature Reviews Genetics. 2015;16(7):421-33.

35. Dong H, Lei J, Ding L, Wen Y, Ju H, Zhang X. MicroRNA: function, detection, and bioanalysis. Chemical reviews. 2013;113(8):6207-33.

36. van Rooij E. The art of microRNA research. Circulation research. 2011;108(2):219-34.

37. Sevignani C, Calin GA, Siracusa LD, Croce CM. Mammalian microRNAs: a small world for fine-tuning gene expression. Mammalian genome : official journal of the International Mammalian Genome Society. 2006;17(3):189-202.

38. Garzon R, Calin GA, Croce CM. MicroRNAs in cancer. Annual review of medicine. 2009;60:167-79.

39. Winter J, Jung S, Keller S, Gregory RI, Diederichs S. Many roads to maturity: microRNA biogenesis pathways and their regulation. Nature cell biology. 2009;11(3):228-34.

40. Ryan BM, Robles AI, Harris CC. Genetic variation in microRNA networks: the implications for cancer research. Nature Reviews Cancer. 2010;10(6):389-402.

41. Mencía Á, Modamio-Høybjoer S, Redshaw N, Morín M, Mayo-Merino F, Olavarrieta L et al. Mutations in the seed region of human miR-96 are responsible for nonsyndromic progressive hearing loss. Nature genetics. 2009;41(5):609-13.
42. Sacheli R, Nguyen L, Borgs L, Vandenbosch R, Bodson M, Lefebvre P et al. Expression patterns of miR-96, miR-182 and miR-183 in the developing inner ear. Gene Expression Patterns. 2009;9(5):364-70.

43. Hughes AE, Bradley DT, Campbell M, Lechner J, Dash DP, Simpson DA et al. Mutation altering the miR-184 seed region causes familial keratoconus with cataract. The American Journal of Human Genetics. 2011;89(5):628-33.

44. de Pontual L, Yao E, Callier P, Faire V, Drouin V, Cariou S et al. Germline deletion of the miR-17 [sim] 92 cluster causes skeletal and growth defects in humans. Nature genetics. 2011;43(10):1026-30.

45. Zhang X, Dong H, Tian Y. miRNA Biology in Pathological Processes. MicroRNA Detection and Pathological Functions. Springer; 2015. p. 7-22.

46. Kawahara Y. Human diseases caused by germline and somatic abnormalities in microRNA and microRNA-related genes. Congenital anomalies. 2014;54(1):12-21.

47. Jiang Q. WY, Hao Y., Juan L., Teng M., Zhang X., Li M., Wang G., Liu Y., iR2Disease: a manually curated database for microRNA deregulation in human disease. Nucleic Acids Res :D98-104. 2009;37: D98-104

48. Li Y, Kowdley KV. MicroRNAs in common human diseases. Genomics Proteomics Bioinformatics. 2012;10(5):246-53.

49. Sessa R, Hata A. Role of microRNAs in lung development and pulmonary diseases. Pulmonary circulation. 2013;3(2):315-28.

50. Angulo M, Lecuona E, Sznajder JI. Role of MicroRNAs in lung disease. Archivos de Bronconeumología (English Edition). 2012;48(9):325-30.

51. Tomankova T, Petrek M, Kriegova E. Involvement of microRNAs in physiological and pathological processes in the lung. Respiratory research. 2010;11:159.

52. Rodriguez A, Vigorito E, Clare S, Warren MV, Couttet P, Soond DR et al. Requirement of bic/microRNA-155 for normal immune function. Science. 2007;316(5824):608-11.

53. Wang Y, Weng T, Gou D, Chen Z, Chintagari NR, Liu L. Identification of rat lung-specific microRNAs by microRNA microarray: valuable discoveries for the facilitation of lung research. BMC genomics. 2007;8(1):29.

54. Mendell JT. miRiad roles for the miR-17-92 cluster in development and disease. Cell. 2008;133(2):217-22.
55. Lu Y, Thomson JM, Wong HYF, Hammond SM, Hogan BL. Transgenic over-expression of the microRNA miR-17-92 cluster promotes proliferation and inhibits differentiation of lung epithelial progenitor cells. Developmental biology. 2007;310(2):442-53.

56. Thomson JM, Parker J, Perou CM, Hammond SM. A custom microarray platform for analysis of microRNA gene expression. Nature methods. 2004;1(1):47-53.

57. Hayashita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S et al. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer research. 2005;65(21):9628-32.

58. Abd-El-Fattah AA, Sadik NAH, Shaker OG, Aboulftouh ML. Differential microRNAs expression in serum of patients with lung cancer, pulmonary tuberculosis, and pneumonia sarcoidosis Cell biochemistry and biophysics. 2013;67(3):875-84.

59. Hemida MG, Ye X, Thair S, Yang D. Exploiting the Therapeutic Potential of MicroRNAs in Viral Diseases. Molecular diagnosis & therapy. 2010;14(5):271-82.

60. Mallick B, Ghosh Z, Chakrabarti J. MicroRNome analysis unravels the molecular basis of SARS infection in bronchoalveolar stem cells. PloS one. 2009;4(11):e7837.

61. Huang W, Li MD. Differential allelic expression of dopamine D1 receptor gene (DRD1) is modulated by microRNA miR-504. Biological psychiatry. 2009;65(8):702-5.

62. Wang G, Wang R, Strulovici-Barel Y, Salit J, Staudt MR, Ahmed J et al. Persistence of smoking-induced dysregulation of miRNA expression in the small airway epithelium despite smoking cessation. PloS one. 2015;10(4):e0120824.

63. Momi N, Kaur S, Rachagani S, Ganti AK, Batra SK. Smoking and microRNA dysregulation: a cancerous combination. Trends in molecular medicine. 2014;20(1):36-47.

64. Wei Shi, Felicia Chen, and Wellington V. Cardoso. Mechanisms of Lung Developme
1. Contribution to Adult Lung Disease and Relevance to Chronic Obstructive Pulmonary Disease

2. Proc Am Thorac Soc. 2009 Dec 1; 6(7): 558–563.

65. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. The New England journal of medicine. 2004;350(26):2645-53.

66. Ezzie ME, Crawford M, Cho JH, Orellana R, Zhang S, Gelinas R et al. Gene expression networks in COPD: microRNA and mRNA regulation. Thorax. 2012;67(2):122-31.

67. Graff JW, Powers LS, Dickson AM, Kim J, Reisetter AC, Hassan IH et al. Cigarette smoking decreases global microRNA expression in human alveolar macrophages. PloS one. 2012;7(8):e44066. 68. O'Leary SM, Coleman MM, Chew WM, Morrow C, McLaughlin AM, Gleeson LE et al. Cigarette smoking impairs human pulmonary immunity to Mycobacterium tuberculosis. American journal of respiratory and critical care medicine. 2014;190(12):1430-6.

68. Wang R, Li M, Zhou S, Zeng D, Xu X, Xu R et al. Effect of a single nucleotide polymorphism in miR-146a on COX-2 protein expression and lung function in smokers with chronic obstructive pulmonary disease. International journal of chronic obstructive pulmonary disease. 2015;10:463.

69. Sato T, Liu X, Nelson A, Nakanishi M, Kanaji N, Wang X et al. Reduced miR-146a increases prostaglandin E2 in chronic obstructive pulmonary disease fibroblasts. American journal of respiratory and critical care medicine. 2010;182(8):1020-9.

70. Cornett AL, Lutz CS. Regulation of COX-2 expression by miR-146a in lung cancer cells. rna. 2014;20(9):1419-30.

71. Van Pottelberge GR, Mestdagh P, Bracke KR, Thas O, van Durme YM, Joos GF et al. MicroRNA expression in induced sputum of smokers and patients with chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 2011;183(7):898-906.

72. Swallow EB, Reyes D, Hopkinson NS, Man WD, Porcher R, Cetti EJ et al. Quadriceps strength predicts mortality in patients with moderate to severe chronic obstructive pulmonary disease. Thorax. 2007;62(2):115-20.
73. Maltais F, LeBlanc P, Jobin J, Casaburi R. Peripheral muscle dysfunction in chronic obstructive pulmonary disease. Clinics in chest medicine. 2000;21(4):665-77.

74. Gea J, Agusti A, Roca J. Pathophysiology of muscle dysfunction in COPD. Journal of Applied Physiology. 2013;114(9):1222-34.

75. Lewis A, Riddoch-Contreras J, Natanek SA, Donaldson A, Man WD, Moxham J et al. Downregulation of the serum response factor/miR-1 axis in the quadriceps of patients with COPD. Thorax. 2012;67(1):26-34. doi:10.1136/thoraxjnl-2011-200309.

76. Locksley RM. Asthma and allergic inflammation. Cell. 2010;140(6):777-83. doi:10.1016/j.cell.2010.03.004.

77. Kumar A, Ghosh B. Genetics of asthma: a molecular biologist perspective. Clin Mol Allergy. 2009;7(7):7.

78. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nature medicine. 2012;18(5):716-25.

79. Wu X-B, Wang M-Y, Zhu H-Y, Tang S-Q, You Y-D, Xie Y-Q. Overexpression of microRNA-21 and microRNA-126 in the patients of bronchial asthma. International journal of clinical and experimental medicine. 2014;7(5):1307.

80. Lu TX, Munitz A, Rothenberg ME. MicroRNA-21 is up-regulated in allergic airway inflammation and regulates IL-12p35 expression. The Journal of Immunology. 2009;182(8):4994-5002.

81. Sawant D, Yao W, Wright Z, Sawyers C, Tepper R, Gupta S et al. Serum MicroRNA-21 as a Biomarker for Allergic Inflammatory Disease in Children. MicroRNA (Shariqah, United Arab Emirates). 2015.

82. Perry MM, Adcock IM, Chung KF. Role of microRNAs in allergic asthma: present and future. Current opinion in allergy and clinical immunology. 2015;15(2):156-62.

83. Collison A, Herbert C, Siegle JS, Mattes J, Foster PS, Kumar RK. Altered expression of microRNA in the airway wall in chronic asthma: miR-126 as a potential therapeutic target. BMC pulmonary medicine. 2011;11:29. doi:10.1186/1471-2466-11-29.

84. Lu TX, Hartner J, Lim E-J, Fabry V, Mingler MK, Cole ET et al. MicroRNA-21 limits in vivo immune response-mediated activation of the IL-12/IFN-γ
pathway, Th1 polarization, and the severity of delayed-type hypersensitivity.
The Journal of Immunology. 2011;187(6):3362-73.
85. Chiba Y, Misawa M. MicroRNAs and their therapeutic potential for human diseases: MiR-133a and bronchial smooth muscle hyperresponsiveness in asthma. Journal of pharmacological sciences. 2010;114(3):264-8.
86. Jazwa A, Kasper L, Bak M, Sobczak M, Szade K, Jozkowicz A et al. Differential Inflammatory MicroRNA and Cytokine Expression in Pulmonary Sarcoidosis. Archivum immunologicae et therapiae experimentalis. 2015;63(2):139-46.
87. Crouser ED, Julian MW, Crawford M, Shao G, Yu L, Planck SR et al. Differential expression of microRNA and predicted targets in pulmonary sarcoidosis. Biochemical and biophysical research communications. 2012;417(2):886-91.
88. Maertzdorf J, Weiner J, Mollenkopf H-J, Network T, Bauer T, Prasse A et al. Common patterns and disease-related signatures in tuberculosis and sarcoidosis. Proceedings of the National Academy of Sciences. 2012;109(20):7853-8.
89. Tarver T. Cancer Facts & Figures 2012. American Cancer Society (ACS) Atlanta, GA: American Cancer Society, 2012. 66 p., pdf. Available from. Journal of Consumer Health on the Internet. 2012;16(3):366-7.
90. Cherni I, Weiss GJ. miRNAs in lung cancer: large roles for small players. Future Oncol. 2011;7(9):1045-55. doi:10.2217/fon.11.74.
91. Leidinger P, Keller A, Meese E. MicroRNAs—important molecules in lung cancer research. Frontiers in genetics. 2011;2.
92. Esquela-Kerscher A, Trang P, Wiggins JF, Patrawala L, Cheng A, Ford L et al. The let-7 microRNA reduces tumor growth in mouse models of lung cancer. Cell cycle. 2008;7(6):759-64.
93. Karube Y, Tanaka H, Osada H, Tomida S, Tatematsu Y, Yanagisawa K et al. Reduced expression of Dicer associated with poor prognosis in lung cancer patients. Cancer science. 2005;96(2):111-5.
94. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nature Reviews Cancer. 2006;6(11):857-66.
95. Liu X, Sempere LF, Ouyang H, Memoli VA, Andrew AS, Luo Y et al. MicroRNA-31 functions as an oncogenic microRNA in mouse and human lung
cancer cells by repressing specific tumor suppressors. The Journal of clinical investigation. 2010;120(4):1298.

96. He L, He X, Lim LP, De Stanchina E, Xuan Z, Liang Y et al. A microRNA component of the p53 tumour suppressor network. Nature. 2007;447(7148):1130-4.

97. Crawford M, Batte K, Yu L, Wu X, Nuovo GJ, Marsh CB et al. MicroRNA 133B targets pro-survival molecules MCL-1 and BCL2L2 in lung cancer. Biochemical and biophysical research communications. 2009;388(3):483-9.

98. Nasser MW, Datta J, Nuovo G, Kutay H, Motiwala T, Majumder S et al. Down-regulation of micro-RNA-1 (miR-1) in lung cancer suppression of tumorigenic property of lung cancer cells and their sensitization to doxorubicin-induced apoptosis by miR-1. Journal of Biological Chemistry. 2008;283(48):33394-405.

99. Davidson MR, Larsen JE, Yang IA, Hayward NK, Clarke BE, Duhig EE et al. MicroRNA-218 is deleted and downregulated in lung squamous cell carcinoma. PloS one. 2010;5(9):e12560.

100. Markou A, Tsaroucha EG, Kaklamanis L, Fotinou M, Georgoulias V, Lianidou ES. Prognostic value of mature microRNA-21 and microRNA-205 overexpression in non–small cell lung cancer by quantitative real-time RT-PCR. Clinical chemistry. 2008;54(10):1696-704.

101. Seike M, Goto A, Okano T, Bowman ED, Schetter AJ, Horikawa I et al. MiR-21 is an EGFR-regulated anti-apoptotic factor in lung cancer in never-smokers. Proceedings of the National Academy of Sciences. 2009;106(29):12085-90.

102. Zhang J-g, Wang J-j, Zhao F, Liu Q, Jiang K, Yang G-h. MicroRNA-21 (miR-21) represses tumor suppressor PTEN and promotes growth and invasion in non-small cell lung cancer (NSCLC). Clinica Chimica Acta. 2010;411(11):846-52.

103. Jackson AL, Linsley PS. Recognizing and avoiding siRNA off-target effects for target identification and therapeutic application. Nature reviews Drug discovery. 2010;9(1):57-67.

104. Al-Ali HK, Jaekel N, Niederwieser D. The role of hypomethylating agents in the treatment of elderly patients with AML. Journal of geriatric oncology. 2014;5(1):89-105.
105. Trang P, Wiggins JF, Daige CL, Cho C, Omotola M, Brown D et al. Systemic delivery of tumor suppressor microRNA mimics using a neutral lipid emulsion inhibits lung tumors in mice. Molecular Therapy. 2011;19(6):1116-22.

106. Rai K, Takigawa N, Ito S, Kashihara H, Ichihara E, Yasuda T et al. Liposomal delivery of microRNA-7-expressing plasmid overcomes epidermal growth factor receptor tyrosine kinase inhibitor-resistance in lung cancer cells. Molecular cancer therapeutics. 2011;10(9):1720-7.

107. Chen Y, Zhu X, Zhang X, Liu B, Huang L. Nanoparticles modified with tumor-targeting scFv deliver siRNA and miRNA for cancer therapy. Molecular Therapy. 2010;18(9):1650-6.

108. Shi Y, Liu C, Liu X, Tang DG, Wang J. The microRNA miR-34a inhibits non-small cell lung cancer (NSCLC) growth and the CD44hi stem-like NSCLC cells. PloS one. 2014;9(3):e90022.

109. Xue W, Dahlman JE, Tammela T, Khan OF, Sood S, Dave A et al. Small RNA combination therapy for lung cancer. Proceedings of the National Academy of Sciences. 2014;111(34):E3553-E61.

110. Wiggins JF, Ruffino L, Kelnar K, Omotola M, Patrawala L, Brown D et al. Development of a lung cancer therapeutic based on the tumor suppressor microRNA-34. Cancer research. 2010;70(14):5923-30.

111. Craig V, Tzankov A, Flori M, Schmid C, Bader A, Müller A. Systemic microRNA-34a delivery induces apoptosis and abrogates growth of diffuse large B-cell lymphoma in vivo. Leukemia. 2012;26(11):2421-4.

112. Bader AG. miR-34a microRNA replacement therapy is headed to the clinic. Frontiers in genetics. 2012;3:120.

113. Bouchie A. First microRNA mimic enters clinic. Nature biotechnology. 2013;31(7):577-.

114. Trang P, Medina PP, Wiggins JF, Ruffino L, Kelnar K, Omotola M et al. Regression of murine lung tumors by the let-7 microRNA. Oncogene. 2010;29(11):1580-7.

115. Scherr M, Venturini L, Battmer K, Schaller-Schoenitz M, Schaefer D, Dallmann I et al. Lentivirus-mediated antagomir expression for specific inhibition of miRNA function. Nucleic acids research. 2007;35(22):e149-e.

116. Ebert MS, Sharp PA. MicroRNA sponges: progress and possibilities. RNA. 2010;16(11):2043-50.
17. Ørum H. Locked Nucleic Acids as MicroRNA Therapeutics. MicroRNAs in Medicine. 2014:663-72.
18. Chakravarthy MV, Zhu Y, Lopez M, Yin L, Wozniak DF, Coleman T et al. Brain fatty acid synthase activates PPARalpha to maintain energy homeostasis. The Journal of clinical investigation. 2007;117(9):2539-52.
19. Boeri M, Sestini S, Fortunato O, Verri C, Suatoni P, Pastorino U et al. Recent advances of microRNA-based molecular diagnostics to reduce false-positive lung cancer imaging. Expert review of molecular diagnostics. 2015;15(6):801-13.
20. Hassan F, Nuovo GJ, Crawford M, Boyaka PN, Kirkby S, Nana-Sinkam SP et al. MiR-101 and miR-144 regulate the expression of the CFTR chloride channel in the lung. PloS one. 2012;7(11):e50837.
21. Graff JW, Powers LS, Dickson AM, Kim J, Reisetter AC, Hassan IH et al. Cigarette smoking decreases global microRNA expression in human alveolar macrophages. 2012.
22. Mattes J, Collison A, Plank M, Phipps S, Foster PS. Antagonism of microRNA-126 suppresses the effector function of TH2 cells and the development of allergic airways disease. Proceedings of the National Academy of Sciences. 2009;106(44):18704-9.
23. Song C, Ma H, Yao C, Tao X, Gan H. Alveolar Macrophage-Derived Vascular Endothelial Growth Factor Contributes to Allergic Airway Inflammation in a Mouse Asthma Model. Scandinavian journal of immunology. 2012;75(6):599-605.
24. Collison A, Mattes J, Plank M, Foster PS. Inhibition of house dust mite–induced allergic airways disease by antagonism of microRNA-145 is comparable to glucocorticoid treatment. Journal of Allergy and Clinical Immunology. 2011;128(1):160-7. e4.
25. Chiba Y, Tanabe M, Goto K, Sakai H, Misawa M. Down-regulation of miR-133a contributes to up-regulation of Rhoa in bronchial smooth muscle cells. American journal of respiratory and critical care medicine. 2009;180(8):713-9.
26. Schembri F, Sridhar S, Perdomo C, Gustafson AM, Zhang X, Ergun A et al. MicroRNAs as modulators of smoking-induced gene expression changes in human airway epithelium. Proceedings of the National Academy of Sciences. 2009;106(7):2319-24.
127. Mayoral RJ, Deho L, Rusca N, Bartonicek N, Saini HK, Enright AJ et al. MiR-221 influences effector functions and actin cytoskeleton in mast cells. 2011.
128. Sharma A, Kumar M, Ahmad T, Mabalirajan U, Aich J, Agrawal A et al. Antagonism of mmu-mir-106a attenuates asthma features in allergic murine model. Journal of Applied Physiology. 2012;113(3):459-64.
129. Bhattacharyya S, Balakathiresan NS, Dalgard C, Gutti U, Armistead D, Jozwik C et al. Elevated miR-155 promotes inflammation in cystic fibrosis by driving hyperexpression of interleukin-8. Journal of Biological Chemistry. 2011;286(13):11604-15.
130. Lin PY YS, Yang PC. MicroRNA in lung cancer. Br J Cancer. 2010;103:1144-8.
131. Liu X, Sempere LF, Guo Y, Korc M, Kauppinen S, Freemantle SJ et al. Involvement of microRNAs in lung cancer biology and therapy. Translational Research. 2011;157(4):200-8.
132. Babu DM, C SS, Shah AP, H GS. Preparation and characterization of poly(2-hydroxyethyl methacrylate-co-methyl methacrylate) hydrogels for sustained delivery of antitumor drug. Current drug delivery. 2011.
133. Barger JF, Nana-Sinkam SP. MicroRNA as tools and therapeutics in lung cancer. Respiratory medicine. 2015;109(7):803-12.
134. Andrade SE, Raebel MA, Morse AN, Davis RL, Chan KA, Finkelstein JA et al. Use of prescription medications with a potential for fetal harm among pregnant women. Pharmacoepidemiology and drug safety. 2006;15(8):546-54.
135. Ahmad A, Ginnebaugh KR, Li Y, Bao B, Gadgeel SM, Sarkar FH. miRNA Targeted Therapy in Lung Cancer. MicroRNA Targeted Cancer Therapy. Springer; 2014. p. 99-114.
136. Bhaskaran K, Douglas I, Forbes H, dos-Santos-Silva I, Leon DA, Smeeth L. Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5·24 million UK adults. The Lancet. 2014;384(9945):755-65.
137. Bhutta MR, Hong KS, Kim BM, Hong MJ, Kim YH, Lee SH. Note: three wavelengths near-infrared spectroscopy system for compensating the light absorbance by water. The Review of scientific instruments. 2014;85(2):026111.
138. Hu B, Ying X, Wang J, Piriayapongsa J, Jordan IK, Sheng J et al. Identification of a tumor-suppressive human-specific microRNA within the FHIT tumor-suppressor gene. Cancer research. 2014;74(8):2283-94.

139. Balakrishnan P, Lee BJ, Oh DH, Kim JO, Hong MJ, Jee JP et al. Enhanced oral bioavailability of dexibuprofen by a novel solid self-emulsifying drug delivery system (SEDDS). European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV. 2009;72(3):539-45.

140. Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E et al. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. Proceedings of the National Academy of Sciences of the United States of America. 2007;104(40):15805-10. d

141. Bandi N, Zbinden S, Gugger M, Arnold M, Kocher V, Hasan L et al. miR-15a and miR-16 are implicated in cell cycle regulation in a Rb-dependent manner and are frequently deleted or down-regulated in non-small cell lung cancer. Cancer research. 2009;69(13):5553-9. 143. Bandi N, Vassella E. miR-34a and miR-15a/16 are co-regulated in non-small cell lung cancer and control cell cycle progression in a synergistic and Rb-dependent manner. Molecular cancer. 2011;10:55.
