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MINIREVIEW

New insights into lung development and diseases: the role of microRNAs[1]

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Abstract: MicroRNAs (miRNAs) are short endogenous noncoding RNA molecules (~22 nucleotides) that can regulate gene expression at the post-transcription level. Research interest in the role of miRNAs in lung biology is emerging. MiRNAs have been implicated in a range of processes such as development, homeostasis, and inflammatory diseases in lung tissues and are capable of inducing differentiation, morphogenesis, and apoptosis. In recent years, several studies have reported that miRNAs are differentially regulated in lung development and lung diseases in response to epigenetic changes, providing new insights for their versatile role in various physiological and pathological processes in the lung. In this review, we discuss the contribution of miRNAs to lung development and diseases and possible future implications in the field of lung biology.

Key words: microRNA, lung development, lung diseases, epigenetic regulation, diagnostic tools, therapeutic strategies.

Résumé : Les micro-ARN (miARN) sont de courtes molécules d'ARN endogènes non codantes (~22 nucléotides) qui peuvent réguler l'expression génique au niveau post-transcriptionnel. Le rôle des miARN dans la biologie pulmonaire suscite un intérêt nouveau en recherche. Les micro-ARN ont été impliqués dans un spectre de processus comme le développement, l'homéostasie et l'inflammation des tissus pulmonaires, et ils sont capables d'induire la différenciation, la morphogenèse et l'apoptose. Au cours des dernières années, plusieurs études ont rapporté que les miARN sont régulés de manière différentielle dans le poumon en développement et dans la maladie pulmonaire en réponse à des changements épigénétiques, fournissant un nouvel aperçu de leur rôle versatile dans différents processus physiologiques et pathologiques dans le poumon. Dans cet article de revue, les auteurs discutent de la contribution des miARN au développement pulmonaire et aux maladies pulmonaires, et des possibles implications futures dans le domaine de la biologie pulmonaire. [Traduit par la Rédaction]

Mots-clés : micro ARN, développement pulmonaire, maladies pulmonaires, régulation épigénétique, outils diagnostiques, stratégies thérapeutiques.

Introduction

MiRNAs are highly conserved single-stranded RNA molecules (~22 nucleotides) derived from endogenous transcripts regulating gene expression. MiRNAs regulate mRNA (mRNA) translation or degradation via partial complementarity with the 3′-untranslated region (UTR) of target mRNAs (Seitz et al. 2003) and thereby repress gene expression (Pillai et al. 2005). Mature miRNAs are processed by cleavage of endogenous primary RNA transcripts (pri-miRNA) through RNA polymerase II/III enzymes (Hutvagner and Zamore 2002; Lund et al. 2004). MiRNAs (Ambros 2004) have been implicated in many biological processes associated with development and cell differentiation (Boehm and Slack 2005; Chen et al. 2012; Costinean et al. 2006; Xu et al. 2004; Esau et al. 2004). To date, more than 2000 human miRNAs have been reported in the literature for their significant roles in various diseases (Sanger miRBase version 21, http://www.mirbase.org/blog/2014/06/mirbase-21-finally-arrives/).

The functional relevance of miRNAs to lung development in humans is lacking. This is possibly due to: (*i*) limited knowledge about the molecular signaling that involves interactions between miRNAs and their targets in the lung microenvironment throughout development, (*ii*) lung-specific transcription factors contributions to signaling cascades that regulate alveolar growth and morphogenesis in normal and pathological lung development are poorly understood, and (*iii*) sparse functional data confirming the epigenetic regulation that may alter miRNA-mediated regulation of gene expression programs. Limited information is available regarding the regulation of miRNA expression and their potential role in lung pathophysiology, despite several studies addressing the involvement of miRNAs in lung development and diseases (Nana-Sinkam et al. 2009; Tomankova et al. 2010; Oglesby et al. 2010; Sayed and Abdellatif 2011; Khoshgoo et al. 2013). Therefore, understanding the miRNA regulatory network and their physiological role in lung development and diseases can provide new insights into lung biology. This review focuses on the role of miRNAs in lung biology and their potential contribution to lung development, homeostasis and inflammatory diseases.

MiRNA biogenesis and biological function

MiRNAs are derived from introns of protein-coding genes or exons of noncoding genes of the genome. Figure 1 represents a

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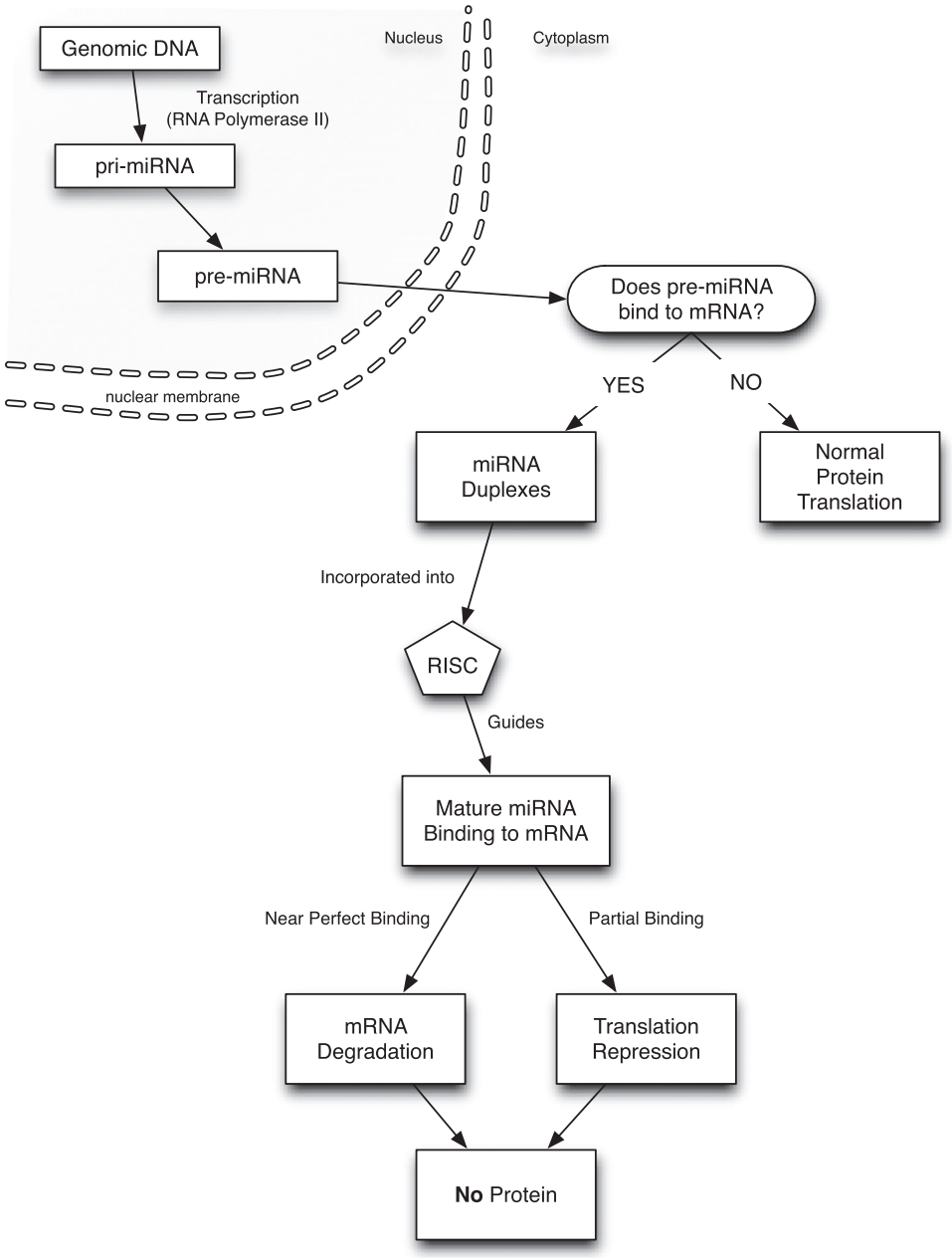
Fig. 1. Schematic representation of miRNA biogenesis pathways. Shown are two pathways for miRNA function: degradation of mRNA and normal mRNA function.



schematic overview of miRNA biogenesis. Briefly, miRNAs are transcribed from primary miRNAs (pri-miRNAs) in two stages. The first step involves the nuclear cleavage of the pri-miRNA by RNA polymerase II. Subsequently, this liberates stem loop-like structures (60–70 nucleotides) known as the miRNA precursor, or the pre-miRNA. The Drosha-DGCR8 complex regulates this step. Next, the precursor of a mature miRNA (pre-miRNA) is actively transported from the nucleus to the cytoplasm by the GTP-binding nuclear protein Ran through the help of Exportin-5 (Yi et al. 2003; Lund et al. 2004). The second step involves the formation of a miRNA duplex in the cytoplasm by Drosha from pre-miRNA to mature miRNA (Kim 2005). One of the strands of the miRNA duplex ("guide" strand) is incorporated into the RNA-induced silencing complex (RISC) and functions to guide the RISC complex to its targets, whereas the other strand ("passenger" strand) of the miRNA duplex with short half-life is degraded (Ambros 2004). Based on the degree of complementarity between the miRNA to 3′UTR of target mRNAs, the mature miRNA negatively regulates the gene expression by two mechanisms: (*i*) degradation of mRNA and (*ii*) inhibition of translation initiation. Degradation of the mRNA occurs based on the perfect complementarity between miRNA with target mRNA (Giraldez et al. 2006; Wu et al. 2006). Secondly, translation repression occurs if the target mRNA has partial complementarity to the miRNA (Wightman et al. 1993; Olsen and Ambros 1999; Doench and Sharp 2004). MiRNAs can also be transported to different cells providing an interesting mechanism for intercellular regulation and cross-talk between the cells (Hergenreider et al. 2012; Chen et al. 2012). This suggests that miRNAs can influence the gap junctional intercellular communication (GJIC) between proliferation and differentiation during lung development. However, the regulatory mechanism of miRNAs through GJIC in lung development is poorly understood.

Noncoding regions of the genome can regulate the activity of miRNAs. These noncoding RNA transcripts (transcription products of pseudogenes) have multiple binding sites attracting specific miRNAs to direct target mRNA repression (Salmena et al. 2011; Karreth et al. 2011). Recently, Rutnam and co-workers (2014) investigated the functional role of noncoding RNA molecules (ncRNA) in breast cancer cell lines to understand the regulatory role of pseudogenes on gene expression. These studies showed overexpression of tumour suppressor candidate-2 pseudogene (TUSC2P) and the TUSC2 3′-UTR in breast cancer cell lines results in downregulation of various cell physiological activities regulating miRNA function. Further, they have demonstrated that the overexpression of 3′-UTR can modulate the endogenous miRNA role (Rutnam et al. 2014) in promoting the translation of TUSC2 function. Limited information is available with respect to the role of long noncoding RNAs (lncRNAs) function in lung development. LncRNAs are expressed in the lung and are involved in a variety of biological roles, including differentiation and proliferation (Askarian-Amiri et al. 2011; Cabili et al. 2011; Kretz et al. 2012). However, the mechanisms underlying lncRNA regulation in lung development and diseases are still unknown.

MiRNA regulation of proliferation and differentiation in embryonic lung tissues

Lung development extends from the embryonic period through the fetal period up to birth and afterwards. Human lung buds form in the first four weeks of the foetus development by signals from the mesenchyme, including fibroblast growth factors. During the pseudoglandular stage of organogenesis, week five through sixteen, these cues form the bronchial tree and parts of the parenchyma. This is followed by a differentiation phase, characterised by three stages, the canalicular, to the saccular, and the alveolar stage. During the canalicular stage, week 16 through 24, the lung periphery fully forms, epithelial cells differentiate, and the air-blood barrier starts to build up. In the saccular stage, week 24 through 36, air spaces expand and surfactant forms. Lastly, active alveolar formation via secondary septation occurs throughout the alveolar stage, 36 weeks of gestation till birth and afterwards. The distal tips of the embryonic lung accommodate undifferentiated progenitors that differentiate into a versatile range of specialized epithelial cells. All cellular differentiation programs require the cell to exit the cell cycle at some stage and turn some genes on and other genes off. Apoptosis (programmed cell death) is fundamental to many cellular processes including differentiation. To proliferate, lung cells must escape apoptosis and vice versa. MiRNAs play a significant role in various cell physiological processes and contribute to pulmonary diseases and lung development (Khoshgoo et al. 2013; Herriges and Morrisey 2014; Sessa and Hata 2013; Williams et al. 2007; Bhaskaran et al. 2009; Dong et al. 2010). In normal development, the translational machinery is controlled by a number of miRNA families.

Lu et al. (2007) addressed the function of miRNAs during the late canalicular stage of lung development. They compared the expression profiles of microRNAs between E11.5 (pseudoglandular stage) and E17.5 (late canalicular stage) in mice. MiR-17 was highly expressed at E11.5, while at E17.5 let-7 was the most abundant miRNA (Lu et al. 2008). The miR-17-92 family can regulate cell survival and proliferation in early and late stages of lung development; for example, miR-17-92 deficient mice show lung-related embryonic lethality (Yanaihara et al. 2006; Navarro et al. 2009). MiR-17-92 family also has been implicated in lung cancer via activation of the pro-apoptotic protein Bim and loss of B cell lineage (Lu et al. 2007; Carraro et al. 2009; Ventura et al. 2008). Hayashita et al. (2005) demonstrated that the overexpression of miR-17-92 could lead to the proliferation of lung epithelial progenitors in lung cancer cells.

Less attention has been given to signaling that regulates growth of progenitor cells and their lineages or fates in the epithelium or mesenchyme before or after birth. Such research is particularly important for lung regeneration after damage or insufficient growth. Ras, downstream of tyrosine kinase fibroblast growth factor receptor (FGFR) signaling, has been identified as a potential target of the miR-17-92 cluster, allowing progenitor self-renewal and eliminating differentiation programs in the adult lung (White et al. 2006). Whether the effect of mir-17-92 on progenitor expansion is cell type-specific or multicellular requires more investigation.

Animal models and the role of miRNAs in lung development

Animal models demonstrate that miRNAs are involved in lung development, and that dysregulation of miRNAs can lead to postnatal diseases. Lu et al. (2007) addressed the importance of the specific miRNA-processing proteins Argonaute 1-4 and Dicer in developing lung epithelium and mesoderm. Functional defects of Dicer in lung epithelium can lead to poor branching of airways in lung development, suggesting a regulatory role for miRNAs in lung morphogenesis (Harris et al. 2006). In the lung epithelial branching region during embryonic stage 11.5 (E11.5), Ago1 and Ago2 are highly expressed in epithelial and mesenchymal regions, demonstrating the pivotal role of miRNA regulation in lung remodeling (Lu et al. 2005). Bhaskaran et al. (2009) reported the differential expression of 27 miRNAs at different phases of lung development. For example, miR-29a has been shown to limit lung proliferation during development (Bhaskaran et al. 2009), and over-expression in lung cancer cells appears to inhibit the tumorigenicity (Fabbri et al. 2007).

In recent years, more attention has been given to the regulatory role of the miR-17-92 locus in lung development. For instance, the miRNAs in this locus are overexpressed in undifferentiated lung progenitor epithelial cells, regulating the key cell cycle gene Retinoblastoma-like 2 (RBl2) for cell proliferation (Lu et al. 2007, 2008). Differential expression of miR-127 and miR-351 has been observed in the saccular–alveolar region of the mesenchymal zone and later expressed in lung epithelial cells, providing a regulatory role for mesenchymal to epithelial transition (Bhaskaran et al. 2009). MiR-17, miR-20a, and miR-106b were demonstrated to regulate E-Cadherin expression in epithelium and mesenchyme during the pseudoglandular stage of lung development via targeting STAT3 and MAPK14 downstream of FGF-10-FGFR-2b cues and consequently modulate timing of sprouty-induced bud morphogenesis (Carraro et al. 2009). Taking into account the aforementioned studies in lung development, miRNAs are believed to participate in a complex regulatory circuit that allows rapid and dynamic lung developmental changes from proliferation to differentiation. The above examples provide an overview of expression profiles of miRNAs between mouse and human lungs hinting at the evolutionary conserved roles of miRNAs during lung development process.

Epigenetic regulation of miRNAs in lung development

Epigenetic regulation involves a series of heritable changes that control phenotype and gene expression without altering the DNA sequence. In recent years, the importance of miRNAs in lung development has emerged, but the genetic mechanism that controls the expression of miRNAs in lung is poorly understood (Herriges and Morrisey 2014). Histone modifications control lung development and gene regulation through epigenetic mechanisms that involve primarily histone acetylation (Jones and Takai 2001; Choudhary et al. 2009). These mechanisms operate through histone acetyltransferase (HAT) and histone deacetylases (HDACs) enhancing transcription and later by silencing the gene function

Table 1. Epigenetic regulation of miRNAs in lung development.

miRNA	Target genes	Function	References
miR-302/367	GATA6	Controls lung endoderm progenitor proliferation, differentiation and apical-basal polarity. The cluster also plays a role in lung homeostasis.	(Tian et al. 2011; Zhang et al. 2008).
miR-17–92 family	RAB14, BMP4, FGF-10, DNMT-1	Regulates early lung development by promoting proliferation and inhibiting differentiation of lung epithelium.	(Carraro et al. 2009; Dakhlallah et al. 2013; Herriges and Morrisey 2014; Khoshgoo et al. 2013; Lu et al. 2007)
miR-34/449	CCNE1, CCND2, E2F1	Role in the development of multiciliogenesis and inhibits lung proliferation through p53 (Trp53) acetylation and activation. The cluster also regulates airway epithelial cells.	(Lize et al. 2010*a*, 2010*b*; Marcet et al. 2011)
miR-375	FZD8	Controls epithelial cell differentiation through WNT/β-catenin pathway.	(Wang et al. 2013*a*)
miR-200 and 205	ZEB1, ZEB2	Role in epigenetic silencing by promoting epithelial-to-mesenchymal transition (EMT) process in human lung epithelial cells.	(Tellez et al. 2011).
let-7 family	RAS, KRAS	Role in sacculo-alveolar stage of lung development and promotes lung tumor development.	(Khoshgoo et al. 2013; Johnson et al. 2005; Kumar et al. 2008)

through a histone tail. Histone acetylation also controls protein function through epigenetic factors (Choudhary et al. 2009) that regulate lung pathologies such as chronic obstructive pulmonary disease (COPD) and asthma (Ito et al. 2002; Banerjee et al. 2012). Wang et al. (2013*b*) determined the activity of HDAC1 and HDAC2 in promoting lung proliferation and airway differentiation through Bmp4/Rb pathways. Further, decreased activity of HDAC1/2 during neonatal development leads to improper alveolarization causing hypoxia and hypoplasia (Londhe et al. 2011; Zhu et al. 2012). Although the functional role of miRNAs regulating histone acetylation in lung development is not clearly understood, epigenetic complexes might play a significant role in lung development. For example, during hypoxia, methyltransferases Suv39H1 and Suv39H2 induce transcriptional silencing via histone H3 lysine 9 methylation, causing repression of gene expression with reduced surfactant protein SP-A expression (Benlhabib and Mendelson 2011), suggesting the regulatory role of methyltransferases during early lung development. Dakhlallah et al. (2013) reported the repression of miR-17-92 cluster function in lung development through DNA methylation by DNA methyltransferase (DNMT)-1 in pulmonary fibrosis.

MiR-10a contributes to multiple cellular processes such as differentiation, apoptosis (Woltering and Durston 2008; Tzur et al. 2008; Ovcharenko et al. 2007), cell survival, replication, and senescence in embryonic development (Bartel 2009; Noren Hooten et al. 2010). MiR-24 and miR-10a target genes have been demonstrated to inhibit endodermal differentiation of human embryonic stem cells (hESC) (Tzur et al. 2008). Developmental regulatory circuitry of hESCs work synergistically with miR-10a in modulating smooth muscle cell differentiation from ESC by repressing histone deacetylase 4 (HDAC4) and thus preventing its undesirable vascular effects in pathologies, such as hypertension (Huang et al. 2010).

Taking the above examples into consideration, it certainly emphasizes that histone acetylation occupies a central role in epigenetic mechanisms regulating lung development and diseases. However, the underlying mechanisms that control these epigenetic factors during lung development and disease states warrant further investigation. In addition, epigenetic factors also influence the regulatory role of miRNAs in lung development, either by promoting or repressing lung proliferation and differentiation. Table 1 represents a list of miRNAs that are epigenetically regulated in lung development.

MiRNAs and lung diseases

MiRNAs are critical for normal lung development and homeostasis. Aberrant expression profiles of certain miRNAs in the lung can impair lung cellular processes and may contribute to lung diseases (reviewed in Table 2). The following section will give insights into miRNA signalling that contributes to the deregulation in lung pathologies.

Lung cancer and bronchopulmonary dysplasia

Lung cancer leads cancer mortality worldwide (Jemal et al. 2009). Some miRNAs function as oncogenes, while others are considered tumor suppressors (Hayashita et al. 2005; Ebi et al. 2009; Liu et al. 2010*b*). To become tumorigenic, epithelial cells need to acquire the ability to migrate and metastasize, a phenomenon called epithelial to mesenchymal transition (EMT). Members of the miR-200 family regulate EMT in lung tumor metastasis. In this regard Pacurari et al. (2013) reported the potential role for the miR-200 family to serve as prognostic markers for non-small cancer lung cells (NSCLC) using H1299 and BEAS-2B cells. Dysregulation of the enzyme Dicer in miRNA biogenesis pathways can also lead to lung tumor development (Bernstein et al. 2003; Kumar et al. 2007). Taking the above examples into consideration, miRNAs might have the potential for new therapy development or as biomarkers for the diagnosis of lung or metastasized cancers. Vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), fibroblast growth factor (FGF), Wnts (Zhang et al. 2012), and sonic hedgehog (SHH) (White et al. 2006) have all been implicated in abnormal signaling that ultimately causes defective gas exchange at birth termed bronchopulmonary dysplasia (BPD). The opposing properties of the angiostatic miR-221 and the angiogenic miR-130a coordinated airway branching in ex vivo mouse fetal lung cultures by altering total VEGFR2-expressing endothelial cells (Mujahid et al. 2013).

Idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease of unknown cause, characterized by interstitial fibrosis impairing lung function. Accumulating evidence suggests that upregulating genes of the TGF-β, SHH, and Wnt/β-catenin signaling cascades advance IPF (Selman et al. 2008; Xu et al. 2003; Fabian et al. 2012). Willis and Borok (2007) reported the involvement of TGF-β and HMGA2 as key players in the pathogenesis of pulmonary fibrosis. Yang et al. (2012*a*) suggested the involvement of the mir-200 family (miR-200a, miR-200b, and miR-200c) in regulating pulmonary fibrosis through EMT. Let-7d has been identified as a key miRNA involved in the pathogenesis of IPF (Pandit et al. 2010). TGF-β-mediated let-7d inhibition triggers an EMT phenotype in patients with IPF. Let-7d, miR-29, and miR-155 all have been implicated in the pathogenesis of pulmonary fibrosis. For example, Let-7 has been shown to suppress genes such as HMGA2 (TGF-β activator),

Table 2. Implications of miRNAs in lung disease.

miRNA	Target genes	Functional role in lung diseases	References
miR-200 family (miR-200b, miR-200a, miR-429, miR-200c and miR-141)	ZEB1, ZEB2, HNRNPR3, HFE ATRX and TGF-β1	Regulates epithelial-mesenchymal transition (EMT) and metastasis in lung cancers.	(Pieraccioli et al. 2013; Li et al. 2014; Pacurari et al. 2013; Yang et al. 2012*a*)
miR-17-92 cluster	PTEN and RB2	Over expressed in human lung cancers and also contributes to uncontrolled inflammation.	(Hayashita et al. 2005; Ventura et al. 2008)
miR-155	SOCS1	Over expression in lung cancer with poor survival rate. Promotes acute lung inflammatory lung injury.	(Rodriguez et al. 2007; Rao et al. 2014)
let-7	RAS	Low expression in human lung cancer with poor prognosis and functions as a tumor suppressor in human lung cells.	(Johnson et al. 2007; Takamizawa et al. 2004)
miR-126	POU3F1, PU.1 and GATA3	Regulates the asthma pathogenesis by altering the immune response of helper T2 cells (TH2). miR-155 elevates the cyclooxygenase expression and PGE2 secretion on in asthmatic airway smooth muscle cells.	(Mattes et al. 2009; Comer et al. 2014)
miR-155	COX2, PGE2		
miR-154	SMAD3, Laminins	Regulates fibroblast migration and proliferation in pulmonary fibrosis.	(Milosevic et al. 2012; Cushing et al. 2011; Yang et al. 2013)
miR-29	Integrins and TGF-β1	Role in the pathogenesis of pulmonary fibrosis.	
miR-424 and 503	APLN, FGF2, BMPR2 and WWP1	Role in the maintenance of pulmonary vascular homeostasis.	(Kim et al. 2013; Yang et al. 2012*b*)
miR-21 SATB1 and YOD1		Role in the pathogenesis of chronic hypoxia-induced pulmonary vascular remodeling.	

KRAS, MYC, cyclins such as D2 and CDK6 (Pandit et al. 2011), implicating the anti-fibrotic role in pulmonary fibrosis.

MiR-21 is expressed in pulmonary myofibroblasts and augments TGF-βR1 signaling, the latter in turn stimulates miR-21-mediated SMAD2/3 phosphorylation and lung fibrosis progression in a feed-forward loop-dependent manner (Liu et al. 2010*a*). Recent studies (Lino Cardenas et al. 2013) indicated that administration of bleomycin in mice triggered symptoms of pulmonary fibrosis by up-regulating miR-199a-5P expression and regulating TGFbeta-induced lung fibrosis by targeting caveolin-1. In early lung development, BMP4 signaling is required for the proliferation of anterior foregut mesenchyme and later in epithelial tube elongation (Weaver et al. 1999; Li et al. 2008). SHH signaling is required for mesenchymal proliferation and specification, lung branching, and smooth muscle cell formation (Litingtung et al. 1998; Radzikinas et al. 2011). BMP and SHH signaling coordinate the separation of the trachea and the esophageal tube (Sala et al. 2011). Radzikinas et al. (2011) demonstrated a mechanism of miR-206-mediated regulation of SHH brain derived neurotrophic factor (BDNF) interaction in airway SMC innervation during airway branching. The study supported the regulatory role of miR-206 by targeting BDNF in early lung bud formation. Manipulating and designing novel therapeutics for specific miRNAs in lung diseases will help us to understand the disease pathogenesis.

Inflammatory airway conditions, cystic fibrosis and asthma

In recent years, many studies have addressed the role of miRNAs in allergic diseases. Lung injury induced by sensitizing cells with aerosolized lipopolysaccharides (LPS) in vitro induces an inflammatory lung condition and dysregulation of a versatile group of miRNAs: miR-146, miR-125b, miR-21, miR-25, miR-27b, miR-100, miR-140, miR-142, miR-3p, miR-181c, miR-187, miR-194, miR-214, miR-223, and miR-224 (Taganov et al. 2006; Tili et al. 2007; Moschos et al. 2007). These findings suggest that these miRNAs are important modulators of lung inflammation that was explained in part by upregulating pro-inflammatory cytokine TNF-α production (Moschos et al. 2007). Interestingly, LPS-mediated regulation of kB-Ras2 also abolished miR-125b effects and blocked IkB signaling (Murphy et al. 2010; Perry et al. 2008; Taganov et al. 2006) during pulmonary inflammation.

The transcription factor Elf5 coordinates embryonic lung cells shape and polarity in response to FGF-10-mediated activation of PI3K/Akt signaling in distal epithelium (Metzger et al. 2008). PI3K/Akt signaling is upregulated in cystic fibrosis (CF), an increased inflammatory condition due to bacterial infections, neutrophil recruitment, and lung tissue breakdown in the airway. Findings suggest that IL-8 expression is upregulated by miR-155-mediated inhibition of phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1 transcription, an event that results in PI3K/Akt signaling activation (Bhattacharyya et al. 2011). Similarly, in airway hyper-responsiveness conditions such as asthma, smooth muscle cells of the lung contract after stimuli resulting in increased cytokine secretion and epithelial hyperplasia. A recent study demonstrated that (Kuo et al. 2013) in an in vitro stretch system, miR-155 expression significantly increased due to the inflammatory response of human bronchial epithelial cells (hBEC) as a result of 24 h mechanical stretch. They demonstrated that the higher expression levels of miR-155 upon hyperstretch repress Src homology 2 domain-containing inositol 5-phosphatase 1 (SH1P1) and promote JNK phosphorylation. MiR-10a regulates PI3K signalling by repressing PI3KCA expression in human airway smooth muscle cells (HASM). PI3KCA inhibition by miR-10a further abrogates AKT phosphorylation and cyclin expression required for HASM proliferation. This suggests that miR-10a potentially has a therapeutic role in controlling the abnormal ASM proliferation (Hu et al. 2014). Dysregulation of miRNAs has been reported in different experimental asthma models. For example, miR-21 deletion in mice challenged with ovalbumin elevates the allergic immune response by repressing IL-12 expression and promoting a Th-2 type lymphocyte response (Lu et al. 2009). Considering smooth muscle cells, miR-133a promotes the expression of RhoA by increasing bronchial hyperactivity in an asthma animal model (Chiba and Misawa 2010). MiR-126 has also been reported to play a key role in the development of allergic airway disease (Mattes et al. 2009). This study found that blocking miR-126 could repress the airway inflammatory disease of the lung by downregulating

the Th2 response that mediates the inflammation. However, the definitive mechanisms that contribute to the Th2 responses in asthmatics remain unclear at this point.

Pulmonary hypertension

Pulmonary hypertension (PH) is a severe disease of pulmonary arteries resulting in right ventricular hypertrophy (RVH), increased fibrosis, and heart failure leading to morbidity and mortality (McLaughlin et al. 2009; Simon 2010). This disease affects primarily pulmonary arterial endothelial cells (PAECs) and pulmonary artery smooth muscle cells (PASMCs). However, the key factors that regulate these events in the pathogenesis of PH are currently unknown. Dysregulation of miRNAs might play a significant role in the development of PH. For example, repression of miR-204 is observed in the PASMCs through STAT3 and SRC kinase (Courboulin et al. 2011). Similarly, miR-424/503 expression is inhibited in PAECs disease by downregulating apelin (APLN) and stimulating proliferation through fibroblast growth factor 2 (FGF2) (Kim et al. 2013). These studies suggest that both APLN and FGF2 play a significant role in mediating the miRNA function in pulmonary vascular homeostasis. MiR-21 and miR-145 occupy a central role in understanding the pathobiology of pulmonary arterial hypertension. For example, downregulation of miR-21 is observed in monocrotaline-injected rats with hypoxia exposure (Caruso et al. 2010). MiR-21 also regulates bone morphogenetic protein (BMP) and Rho/Rho-kinase signaling by repressing RhoB expression and Rho-kinase activation in pulmonary arterial endothelial cells (Parikh et al. 2012; Connolly and Aaronson 2011). This study highlights the central regulatory role for miR-21 in pulmonary hypertension and defines it as a PH-modifying miRNA.

Similarly, miR-21 null mice showed severe disease pathogenesis in pulmonary artery hypertension (PAH) with increased expression of Rho B and Rho-kinase activity (Parikh et al. 2012). MiR-145 is a phenotypic marker for the smooth muscle cells (SMC) regulating the smooth muscle cell function through KLF-5 and myocardin (Cheng et al. 2009; Elia et al. 2009). Repression of miR-145 has been reported to play a role in the re-modelling of vascular homeostasis in mice (Elia et al. 2009). Caruso et al. (2012) have demonstrated that miR-145 inhibition in a mouse model protects against PAH development. This group also demonstrated that the mice exposed to hypoxia exhibits elevated expression of miR-145 and observed this pathological condition in lung tissues from patients with PAH. Further, Fichtlscherer et al. (2010) have identified a role for circulatory miRNAs in coronary artery disease patients, but limited information is available in PH patients. Recently Wei et al. (2013) determined the expression patterns of miRNAs in the blood of pulmonary hypertensive (PH) human subjects and identified novel miRNAs in the circulation of PH subjects. Additionally, they validated and identified miRNAs that were upregulated (miR-23b, miR-130a, and miR-191), or downregulated (miR-451 and miR-1246) in the circulation of PH subjects. These dysregulated miRNAs link directly to the degree of pathogenesis of PH and might be considered as potential biomarkers in PH disease for therapeutic intervention. However, it is still unclear how the dysregulated circulatory miRNAs in the blood stream of PH influence the target genes.

Limitations of lung tissue heterogeneity and available animal models

Two major limitations of existing efforts are the lack of multiple time points in development and the lack of combined analysis of mRNA genes parallel to an analysis of miRNAs. Such factors are important to identify dynamic changes. Available data do not always reflect cell-specific changes in gene expression, since the available studies have been utilizing whole lung tissues, a very heterogeneous and complex organ. Thus, the use of different methods that rely on spatial micro-analysis of lung tissues such as laser capture microdissection or cell sorting is emphasized. Demonstrating such approaches allows for novel markers that will have a significant impact on disease gene–epigenetics interaction discovery. Animal studies on specific miRNA inhibitors or mimics lack clinical efficacy and unlikely trigger exactly the same symptoms in patients. Armed with this challenge, one miRNA could envisage a number of adverse scenarios resulting from indiscriminate targeting of multiple transcripts or pathways. The spatial and temporal regulation that controls such complex crosstalk is obscure in such cases, and the application of one therapy could provoke undesired effects. Moreover, a number of the animal models that are currently available are not fully characterized at the molecular level, and therefore they do not assimilate the players in the human disease. Understanding the molecular mechanisms responsible for miRNA-associated diseases is extremely important. The studies reported to date have only assessed the short-term impact of miRNAs inhibition or overexpression. Although many approaches using miRNAs inhibitors or mimics showed significant results, the constituents used in these studies are not suitable for in vivo miRNA inhibition or ectopic expression. New strategies based on improving penetration, pharmacokinetics, coupling to carrier molecules, antibody conjugates, membrane permeable peptides, colloidal particles, or gene transfer is warranted.

MiRNAs as targets for therapeutic strategy

MiRNAs have been employed as potential therapeutic targets in the treatment of various lung diseases. Recently, molecular strategies have emerged to increase the therapeutic function of miRNAs in diseased tissues. Accordingly, two approaches have been developed for miRNA-based therapeutics: miRNA antagonists and miRNA mimics. MiRNA antagonists inhibit endogenous miRNA function contributing to the gain-of-function in pathological states or inflammatory conditions. This therapeutic approach is compared to other inhibitory therapeutics that target individual gene products as well as short interfering RNAs (siRNAs). MiRNA mimics restore a loss of function and are considered "miRNA replacement therapy", introducing therapeutic miRNAs targeting diseased cells in the patients (Bader et al. 2010). For example, therapeutic delivery of *let*-7 mimic inhibited tumour growth in human non-small cell lung cancer xenografts and in a transgenic mouse model of RAS-G12D (Trang et al. 2010). Similarly, miR-34 mimics therapeutically blocked lung tumour growth in a mouse model (Wiggins et al. 2010).

Anti-microRNA oligonucleotides (*antagomir oligos*) are modified chemically to increase their stability and to silence the miRNAs function in vivo (Krutzfeldt et al. 2005). More research studies are warranted to understand the impact of anti-miR treatment on miRNA–miRNA interaction networks. Recently, more attention has been focused on the locked nucleic acid (LNA) strategy to understand the mechanism of miRNA-mediated gene regulations. Using this strategy, the locked nucleic acid approach has potential in human trials considering their increased specificity versus conventional approaches, theoretically reducing off-target effects (Garzon et al. 2010). For example, LNA antagomirs against miR-33 proved to be beneficial for the treatment of atherosclerosis by regulating the lipoprotein levels (Rayner et al. 2011).

Ample evidence indicates that circulating miRNAs can also serve as biomarkers for disease states, particularly lung cancers. For example, the let-7 family of miRNAs in lung have been shown to regulate several pathways downstream of the RAS oncogene (Johnson et al. 2005). The mutation of this oncogene has been implicated in adenocarcinoma of the lung.

Liu et al. (2010*a*) reported that miR-21 regulates the fibrogenic activity in lung fibrosis through TGF-β1 and Smad/Smad7 pathways. Further, this group addressed the elevated expression of miR-21 in the lungs of mice having lung fibrosis and in patients

with idiopathic pulmonary fibrosis. Similarly, anti-miR-21 has been reported for its therapeutic effects in the treatment of lung fibrosis (Liu et al. 2010*a*). Mir-29 also protects lungs against bleomycin-induced lung fibrosis in mice (Xiao et al. 2012). Mujahid et al. (2013) recently addressed the specific role of anti-angiogenic miR-221 and pro-angiogenic miR-130a effects on airway and vascular development in fetal lungs. These studies have found that lungs treated with anti-miR 221 resulted in increased branching with overexpression of Hoxb5 and VEGFR2 around the airways. Alternatively, miR-221 mimic had opposite effects on treated lungs with respect to airway branching, defining the regulator effects of miR-221 and miR-130a on vascular remodelling of the developing lung. Engineering novel therapeutics against the potential target miR-21 provides better treatment for the pathogenesis of lung fibrosis. Molecular approaches or strategies, applied in miRNA biology to understand the potential interaction between miRNAs and their targets, will guide us to develop effective therapeutics and therapies for the treatment of lung diseases. However, the adverse effects of nonspecific miRNAs inhibitor/mimic-based therapeutics can damage normal proliferating cells. Initial experiments are required to examine such cycling effects on normal cells of the lung microenvironment. This approach is limited by the lack of miRNAs inhibitors/mimics that selectively target the cell cycle of damaged cells or compounds that preferentially arrest normal lung cells at the S phase. These approaches are difficult to exploit clinically.

Conclusion

Lung development is a complicated process integrating cell differentiation, morphogenesis, and regeneration and these processes are controlled, in part, by miRNAs. We have presented a summary on the potential role of miRNAs contributing to lung development and a variety of lung diseases. The insights gained from lung biology research will provide the road map to address the potential role of miRNAs in lung organogenesis and develop novel therapeutics to target specific lung diseases. In the future, pharmaceutical and genetic therapeutic approaches will be employed to examine the role of miRNAs in lung pathobiology. Further, miRNAs could serve as potential targets for lung specific therapy, diagnostic tools for the treatment of various lung diseases, and to develop therapeutics for the novel targets. Thus, understanding the intricacies in miRNA-lung biology will guide us to develop therapeutic strategies and approaches for personalized medicine.

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