Integrating microRNAs into a system biology approach to acute lung injury

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Abstract

Acute lung injury (ALI), including the ventilator-induced lung injury (VILI) and the more severe acute respiratory distress syndrome (ARDS), are common and complex inflammatory lung diseases potentially affected by various genetic and non-genetic factors. Using the candidate gene approach, genetic variants associated with immune response and inflammatory pathways have been identified and implicated in ALI. Since gene expression is an intermediate phenotype that resides between DNA sequence variation and higher level cellular or whole-body phenotypes, the illustration of gene expression regulatory networks could potentially enhance understanding of disease susceptibility and the development of inflammatory lung syndromes. MicroRNAs (miRNAs) have emerged as a novel class of gene regulators which play critical roles in complex diseases including ALI. Comparisons of global miRNA profiles in animal models of ALI and VILI identified several miRNAs (e.g., miR-146a, miR-155) previously implicated in immune response and inflammatory pathways. Therefore, via regulation of target genes in these biological processes and pathways, miRNAs potentially contribute to the development of ALI. While this line of inquiry exists at a nascent stage, miRNAs have the potential to be critical components of a comprehensive model for inflammatory lung disease built by a systems biology approach that integrates genetic, genomic, proteomic, epigenetic as well environmental stimuli information. Given their particularly recognized role in regulation of immune and inflammatory responses, miRNAs also serve as novel therapeutic targets and biomarkers for ALI/ARDS or VILI, thus facilitating the realization of personalized medicine for individuals with acute inflammatory lung disease.

Key Words: microRNA, acute lung injury, mechanical ventilation, inflammation, immune response, gene expression, genomics

Abbreviations: ACE = angiotensin converting enzyme; ALI = acute lung injury; ARDS = acute respiratory distress syndrome; CCR7 = chemokine receptor 7; CXCR3 = chemokine receptor 3; CNV = copy number variant; DHFR = dihydrofolate reductase; eQTLs = expression quantitative trait loci; GADD45A = growth arrest and DNA-damage-inducible, alpha; HTV = high tidal volume; IL6 = interleukin 6; IL-10 = interleukin 10; iNOS = inducible nitric oxide synthase; LBP = lipopolysaccharide binding protein; LPS = lipopolysaccharide; MIF = macrophage migration inhibitory factor; MKP-1 = mitogen-activated protein kinase phosphatase-1; MYLK = myosin light chain kinase 3; PAI-1 = plasminogen activator inhibitor 1; PBEF = pre-B-cell colony enhancing factor; PDCD4 = programmed cell death 4; SNP = single nucleotide polymorphism; SOD3 = extracellular superoxide dismutase; SP-B = surfactant protein B; TGF-β = transforming growth factor β; THBS1 = thrombospondin 1; TLR = toll-like receptors; UTR = untranslated region; VEGF = vascular endothelial growth factor; VILI = ventilator-induced lung injury.

Introduction

Inflammatory lung disorders include a wide range of pulmonary diseases such as cystic fibrosis, asthma, emphysema, chronic bronchitis, and chronic obstructive pulmonary disease, all of which are characterized by increased leukocyte infiltration (e.g., neutrophils and monocytic cells) into lung tissues as the body's immune response to infection or injury. An important inflammatory lung disease of rapid onset is acute lung injury (ALI) and the more severe form of ALI, acute respiratory distress syndrome (ARDS). ALI is a diffuse, heterogeneous type of acute lung injury clinically characterized by progressive hypoxemia, reduced lung compliance, and intense inflammation in the lung tissues that results from either direct injury to the lung (e.g., pneumonia, thoracic trauma, smoke-related lung injury) (1, 2) or an indirect insult (e.g., pancreatitis) (2). It is estimated that each year in the United States alone, there are ~190,000 cases of ALI, which are associated with ~75,000 deaths and ~3.6 million hospital days (2, 3). The treatment of inflammatory lung diseases such as ALI has historically involved supportive care and occasionally use of anti-inflammatory medications, such as corticosteroids, and antibiotics (4). In addition, mechanical ventilation, so critical to support of the critically ill patients with respiratory failure, is recognized to confer additional risk via excessive ventilator-delivered tidal volumes which may worsen preexisting lung injury via shear forces generated during regional delivery of high tidal volume ventilation or even directly induce lung injury in patients placed on mechanical ventilation in the absence of preexisting lung injury (VILI: ventilator-induced lung injury) (5). Another major complication of mechanical ventilation is the development of ventilator-associated pneumonia with both gram-positive and

gram-negative organisms also resulting in subsequent prominent systemic inflammatory response (6). Previous studies have shown that induction of systemic inflammation with intravenous bacterial lipopolysaccharide (LPS) can cause a synergistic increase in lung injury in the setting of mechanical ventilation (7-9).

Among the many unanswered questions regarding ALI and ARDS is the heterogeneous response of individuals to similar risk factors and diseases which can produce ALI. Neither ALI nor ARDS are specific disease processes themselves, but rather represent syndromes developed in a fraction of individuals that are exposed to risk conditions such as trauma, sepsis, and pneumonia which potentially induce ALI (10, 11). A few relationships are obvious, for example, an older patient with serious comorbidities is at greater mortality risk than a younger, healthier patient with the same illness In addition, some important clinical predictors for the development and/or mortality in ALI/ARDS have been identified, including the severity of disease measured by Acute Physiology and Chronic Health Evaluation III score (APACHE), trauma, corticosteroids before ARDS, and packed red blood cell transfusions (12). However, it is well-recognized that despite similar exposures to potential ALI-inciting insults, only a small proportion of exposed individuals will ultimately develop these syndromes (11) and the clinical outcomes in patient with similar clinical characteristics may vary significantly from complete resolution to death. Furthermore, significant race and gender differences have also been reported in the annual ARDS mortality rate (11, 13).

These observations have led to an important focus on the potential contribution of genetics, gene-gene and gene-environment interactions as a potential explanation for the disparities observed in ALI/ARDS patients (11). This review will introduce the general

strategies for identifying genes and genetic variants implicated in ALI, and particularly, the potential role of microRNAs (miRNAs) (Figure 1), a class of novel gene-regulators made of small non-coding RNA molecules, in the pathogenesis of ALI/ARDS and VILI. A picture which is emerging is that miRNAs, through their functional effects on gene regulation, have the potential to regulate many aspects of physiological homeostasis homeostasis (14), modulate the risk of common diseases and drive therapeutic responsiveness (15). Table 1 shows a list of miRNAs recently reported to be implicated in several inflammatory lung diseases. It is anticipated that future miRNA studies will be an important complement to the current genetic, genomic, and proteomic studies in furthering our understanding of common complex diseases including inflammatory lung disorders such as ALI.

Identifying genes and genetic variants implicated in ALI

Studies on the genetic basis of ALI/ARDS and VILI have generally focused on a limited number of putative mechanistic functions in lung injury and/or inflammation (11, 16) (Figure 1). This has identified several well-defined candidate genes such as *ACE* (angiotensin converting enzyme) (17, 18), *SOD3* (extracellular superoxide dismutase) (19, 20), *SP-B* (surfactant protein B) (21, 22), *IL-10* (interleukin 10) (23-25), *VEGF* (vascular endothelial growth factor) (26-28), *PAI-1* (plasminogen activator inhibitor 1) (29), *THBS1* (thrombospondin 1) (30), and *TGF-β* (transforming growth factor β) (31), *MIF* (macrophage migration inhibitory factor) (32), *IL-6* (interleukin 6) (33, 34), *LBP* (lipopolysaccharide binding protein) (35), *MYLK* (myosin light chain kinase) (36-39), *PBEF* (pre-B-cell colony enhancing factor) (40, 41), and *GADD45A* (growth arrest and

DNA-damage-inducible, alpha) (42). The allele frequencies of genetic variants such as those in the form of single nucleotide polymorphisms (SNPs) in these candidate genes as well as their expression patterns were examined between cases and controls (Figure 1). For example, the interest in the relationship between ACE and ARDS originated from the hypothesis that activation of the pulmonary renin-angiotensin system might impact the pathogenesis of ALI/ARDS by inducing apoptosis, altering vascular permeability, vascular tone, and endothelial/ epithelial survival (43). Studies using animal models further suggested that the inflammation and apoptosis in VILI is, at least in part, due to ACE-mediated angiotensin II production (44); and that the inhibition of ACE by captopril (an ACE inhibitor used for the treatment of hypertension and some types of congestive heart failure) may attenuate VILI through the reduction of inflammatory cytokines, inhibition of apoptosis (45) as well as decrease of PAI-1 (46), the elevated expression of which was found to be associated with poor clinical outcomes in both pediatric and adult ALI (47, 48). Furthermore, polymorphisms associated with the gene expression patterns of *ACE*, *PAI-1* and other candidate genes (e.g., *VEGF*, *IL-10*, *SOD3*, *PBEF*) as well as their relationships with lung pathophysiology or clinical characteristics (e.g., mortality) have been identified (11, 16).

Among various phenotypes, gene expression (i.e., mRNA transcript abundance) acts as an intermediate phenotype situated between variation in DNA sequence and other more complex cellular, tissue, organ or whole-body phenotypes. Since gene expression is a quantitative and complex trait that is partially heritable, investigating the regulation of quantitative gene expression phenotypes will be critical to understanding the mechanisms of complex diseases. During the past decade, gene expression alterations have been found

to be implicated in the etiologies of common diseases including cancers, cardiovascular diseases, psychiatric disorders, as well as individual response to therapeutic treatments (49, 50). More recently, comparisons of global gene expression were used to identify differentially expressed genes between cases and controls with the aims to identify more candidate genes in inflammatory lung disease. For example, using the Affymetrix oligonucleotide microarray platform and a canine model of VILI, differential expression levels of a significant number of genes were identified between lung apex/base regions as well as between gravitationally dependent/nondependent regions of the base with major functional groupings of differentially regulated genes including inflammation and immune responses, cell proliferation, adhesion, signaling, and apoptosis (51). In addition to a number of commonly known ALI-associated genes (e.g., *VEGF*, *THBS1*), microarray analysis also revealed several novel genes not previously described in the context of ALI. One such novel gene is *PBEF* that encodes pre-B-cell colony enhancing factor (also known as visfatin), which was subsequently found to be over-expressed in human bronchoalveolar lavage fluid and serum samples from patients with ALI and in cytokine- or cyclic stretch-activated lung microvascular endothelium (16, 41, 52), thus having the potential to be a novel biomarker in ALI (41) .

Gene expression variation and regulation

Gene expression itself is a complex and quantitative phenotype that is regulated by various genetic and non-genetic factors. The contribution of genetics, especially in the form of *cis*- or *trans*-acting single SNPs (i.e., eQTLs, expression quantitative trait loci) has been illustrated using human cell line models (53, 54). For example, studies using the human lymphoblastoid cell line samples (LCLs) (e.g., the International HapMap Project

(55, 56) as well as the original LCLs derived from Caucasians in Utah collected by Centre d'Etude du Polymorphisme Humain) have demonstrated that common genetic variants including SNPs and copy number variants (CNVs) contribute substantially to the variation in gene expression within a population (57-59) and between populations (60-63). Although substantial genetic variations such as SNPs and CNVs partially account for the variation in gene expression observed between individuals and populations (64), a number of other factors including environment (65), epigenetics (e.g., DNA methylation in promoter regions) (66) may also contribute to the dynamic status of gene expression in cells and thus potentially affect individual phenotypes including the susceptibility of ALI/ARDS or VILI. Therefore, investigating the relationships among genetic and non-genetic factors and common diseases may provide us a more comprehensive picture of complex diseases, thus benefiting the ultimate realization of personalized medicine.

Since the discovery of distinct miRNA functions in gene regulation in *C. elegans* almost a decade ago (67), miRNAs have been established to be an important class of gene regulators involved in many biological processes (68). Through their gene regulation functions, miRNAs may provide missing pieces to the understanding of complex traits including the risks of inflammatory lung disease. Naturally, researchers have increasingly focused on the functional relevance and role that miRNAs may play in the pathogenesis of human diseases (Figure 1).

MicroRNAs regulate gene expression

MicroRNAs are a family of small non-coding RNAs (21-25 nucleotides in length) found in almost all metazoan genomes, including worms, flies, plants and mammals (69).

So far, ~700 miRNAs have been identified experimentally or computationally in humans (70-72). As the current cloning technology favors highly expressed miRNAs, more exhaustive cloning efforts may be needed to catalogue all miRNAs (estimated >800 in humans) (73) that must cover more tissue types and developmental stages. Besides cloning, computational prediction was used to identify miRNAs that share homologous sequences with closely related species (73). MiRNA registry databases such as the Sanger Institute miRBase (http://www.mirbase.org/) (70, 71, 74) contains up-to-date annotations for all published miRNAs that were either experimentally validated for mature miRNA expression or computationally predicted for the corresponding hairpin structures. The growing database currently (release 16, September, 2010) contains 1048 distinct mature miRNAs in humans and > 15,000 miRNA sequences in > 140 other species.

MiRNAs have been discovered to play important regulatory roles in gene expression (67). At least for those with characterized gene targets, miRNAs have been found to negatively regulate gene expression at the post-transcriptional level (i.e., through translational repression and mRNA degradation) by binding to the 3' untranslated regions (3'UTRs) (75), although the precise molecular mechanism still needs to be defined. Furthermore, computational predictions of miRNA targets have revealed a variety of regulatory pathways that might be subject to miRNA-mediation regulation (76). Therefore, the emerging picture is that miRNAs, through their roles in gene regulation, have the potential to regulate almost all aspects of physiological conditions including disease susceptibility, disease development as well as response to therapeutic treatments. For example, miRNA alterations or signatures have been found to be involved in the

initiation, progression or prognosis of various complex human diseases such as cancer (77-80), diabetes (81, 82), and cardiovascular disease (83-87).

In addition, a class of functional polymorphisms termed miRSNPs or miRNA polymorphisms were recently reported to be new players in miRNA-mediated gene regulation. The miRSNPs are defined as polymorphisms present at or near miRNA binding sites of functional genes as well as in the genes involved in miRNA biogenesis and in pri-, pre- and mature-miRNA sequences, thus affecting gene expression by interfering with miRNAs (88). For example, the expression of miR-24, a ubiquitously expressed miRNA that has p53-independent tumor suppressor activity, was found to be deregulated in human colorectal tumor through a target site polymorphism (89) . A polymorphism in one of miR-24's target genes, *DHFR* (dihydrofolate reductase), was also found to lead to methotrexate resistance (90). Therefore, detecting miRSNPs would be critical in the studies of molecular epidemiology and the realization of personalized medicine. In fact, a comprehensive analysis of the impact of SNPs and CNVs on human miRNAs and their regulatory genes demonstrated a significant number of SNPs and CNVs in pre-miRNAs and miRNA target genes (91).

MicroRNAs implicated in immune responses and ALI

MicroRNAs have been shown to be centrally involved in the regulation of immune system development, differentiation of B and T cells, proliferation of monocytes and neutrophils, antibody production, the release of inflammatory mediators (92), and certain inflammatory lung diseases (e.g., cystic fibrosis, asthma, and idiopathic pulmonary fibrosis) (Table 1), thus potentially contributing to the pathogenesis of

ALI/ARDS as well. Notably, miRNAs have been found to regulate some well-defined ALI-associated candidate genes (e.g., miR-126 targeting *VEGF*) (93). Taking advantage of the technologies of high throughput miRNA profiling (e.g., the Exiqon MirCURY LNA™ microRNA Array, oligonucleotide miRNA microarray) as well as real-time PCR, we recently sought to determine the role of miRNAs in contributing to immune response in the context of LPS exposure, sepsis or ALI. For example, LPS-induced innate immune response was found to be associated with widespread, rapid and transient increases in miRNA expression in the mouse lung (94). Well-defined relationships between miRNAs and LPS exposure include miR-146a which was found to be NF-κB-dependent and an inhibitor targeting signaling proteins of innate immune response to LPS. miR-146a controls TLRs (toll-like receptors) and cytokine signaling through a negative feedback regulation loop involving down-regulation of IL-1 receptor-associated kinase 1 (IRAK, a recent identified ALI candidate gene) (95) and TNF receptor-associated factor 6 protein levels (96). Similar to miR-146a, the expression of miR-147 appears critical for critical for endotoxin-induced tolerance (97) and is induced in LPS-treated mouse peritoneal macrophages and in the lungs of LPS-exposed mice through the stimulation of multiple TLRs including TLR2, TLR3, and TLR4, which enable inflammatory cells to recognize invading microbial pathogens. In addition, miR-9, which may down-regulate *NFKB1*, was found to be induced in human polymorphonuclear neutrophil and monocytes after TLR4 activation (98). The induction of mR-148 and miR-152 by the activation of TLR3, TLR4, and TLR9 agonists was also found to inhibit the production of cytokines including IL-12, IL-6, TNF- α (99). Thus, a negative feedback loop exists in which TLR stimulation induces miRNAs such as miR-147, miR-9, miR-148, miR-152 to prevent

excessive inflammatory responses is likely (98-100), thus contributing to immune homeostasis and immune regulation. The observations that miR-155 expression was increased in MKP-1 (mitogen-activated protein kinase phosphatase-1)-deficient mouse macrophages while inducing iNOS (inducible nitric oxide synthase) expression suggested its potential role in inflammatory response to LPS challenge (101, 102). The down-regulation of miR-125b by LPS/TNF-α stimulation suggested its possible roles in the response to endotoxin shock (101). More recently, miR-21 was found to regulate *PDCD4* (programmed cell death 4) expression after LPS stimulation. Particularly, transfection of cells with a miR-21 precursor blocked NF-κB activity and promoted IL-10 production in response to LPS, whereas transfection with antisense oligonucleotides to miR-21 had the opposite effect (103). Furthermore, a new mechanism of miRNA-mediated gene regulation (i.e., through competition with RNA-binding protein) was recently identified for miR-466I, which up-regulates *IL-10* expression in TCR-triggered macrophage (104),

In addition, our recent work on *PBEF*, a candidate gene for ALI, using human pulmonary artery endothelial cells suggests that miR-374a and miR-568 may decrease endogenous *PBEF* expression under 24 hrs of LPS challenge (unpublished data), indicating the potential role of miRNAs in regulating these genes in related pathways. Furthermore, we found that miR-516a-5p was down-regulated in human lung microvascular endothelial cells after under LPS and HMW-HA (high molecular weight hyaluronan) challenge for 24 hrs (unpublished data). Interestingly, miR-516a-5p may regulate *BLNK* (B-cell linker) (105), suggesting a putative role of miR-516a-5p in B-cell differentiation and immune response, thus may be implicated in ALI.

Although most studies concerning miRNAs and inflammatory lung disease have been related to LPS responses to date, using animal models of ALI or VILI, there has been efforts to directly identify miRNAs that are differentially expressed. To further understand the mechanisms by which shear forces or cyclic stretch is translated into vascular barrier disruption and inflammation in the development of VILI, the effect of high tidal ventilation (HTV) on lung miRNA expression was studied in a murine model of VILI (106). The expression levels of 365 miRNAs were compared between HTV-treated mice and control mice using TaqMan miRNA microarrays (106) and miRNA expression found to be altered according to the length of HTV treatment, suggesting a direct inducing role of VILI on miRNA expression. For example, the expression levels of 13 miRNAs were decreased by at least 50% after 4 hours of HTV, and in 12 of these differential miRNAs, a decrease of at least 30% was apparent after only one hour of HTV (106). Several of these miRNAs were known to be associated with regulation of inflammation (106) and suggest that miRNAs may play an important role in lung inflammation associated with VILI and thus provide a potentially novel therapeutic target for VILI. It should be noted that the alterations of miRNAs in VILI lungs could come from infiltrated cells. Therefore, further studies may be necessary to comprehensively evaluate these observations.

MicroRNAs as potential molecular therapies and biomarkers in ALI

Currently, although the annual ARDS mortality rate is very slowly declining (107), given that ALI is a common disease (~200,000 cases annually in the United States)

and a mortality rate that exceeds 35% (11), there are compelling reasons to further our mechanistic understanding of ALI/ARDS and VILI. With the accumulating evidence that changes in miRNA expression are associated with immune response, inflammation pathways, and the pathogenesis of inflammatory lung disease including ALI, the potential of targeting miRNAs as a novel therapeutic approach looks very promising. The main molecular alterations in miRNAs are represented by gene expression variation (67). Although gene expression variation produced by miRNAs are usually moderate, but the consequence could potentially affect a vast number of target genes (70), which in turn may influence various physiological pathways. RNA inhibition technologies using miRNAs may be used to block mRNA production and/or function of disease-related genes. For example, miRNAs that are negatively associated with the expression of inflammation pathway genes could be used to avoid over-expression of cytokines to repress acute inflammatory response in patients under mechanical ventilation, thus helping prevent the occurrence of VILI. In addition, miRNAs that directly participate in the pathogenesis of ALI/ARDS or VILI could be targeted by antisense oligonucleotides (i.e., antagomirs) by taking advantage of the sequence structure of these small RNA molecules (108).

The expression patterns of miRNAs are believed to be dynamic and reflect the changing intra-/extra-cellular environment and signals. Therefore, miRNAs could be attractive as potential biomarkers to represent the underlying pathophysiological processes in specific disease states or development stages. Moreover, miRNAs can be detected in a variety of sources, including tissue, blood and body fluids. Also, they are reasonably stable and appear to be resistant to differences in sample handling, which

increases their appeal as practical biomarkers (109). Recently, miRNAs were identified in serum and plasma as biomarkers for diagnosing and monitoring several diseases including cancer, cardiovascular diseases, rheumatic diseases (109-111). Circulating miR-146a and miR-223 were found significantly reduced in septic patients compared between systemic inflammatory response syndrome patients and healthy controls (112). Wang et al. showed that serum miR-146a and miR-223 might serve as new biomarkers for sepsis with high specificity and sensitivity (112). In contrast, Vsilescu et al. reported that the expression of miR-150 correlated with the aggressiveness of sepsis, therefore, they suggested that miR-150 could be plasma prognostic marker in patients with sepsis (113). With the expected advances in understanding the relationships between miRNAs and ALI/ARDS or VILI through genome-wide miRNA profiling, more miRNA biomarkers associated with immune response, inflammation pathways, disease development, and disease severity could be evaluated as a novel tool in the diagnosis and monitor for inflammatory lung disease.

Future perspectives

Gene expression, a quantitative and complex trait, has been extensively studied during the past few years, especially using the human cell line models and the HapMap genotypic data (53). Genetic variants like SNPs and CNVs have been found to contribute substantially to gene expression variation (57-63). Defining the roles of miRNAs in gene expression regulation, however, still have many important hurdles to cross. Before we could comprehensively understand the role of miRNAs in inflammatory lung disease, some basic research studies on their role in gene regulation (e.g., building a systematic

and more reliable catalogue of miRNA gene targets) will prove to be critical and benefit the research community. For example, due to cost and efficiency, current miRNA target identification still relies largely on computational algorithms (e.g., miRanda used by the miRBase (71, 74), TargetScan (114, 115), PicTar (116)) that aim to take advantage of the biochemical/thermodynamic properties of the sequences of miRNAs and their gene targets. Although successful to some extent, the prediction results of these computational methods are generally uncorrelated and their predictions are often not supported by each other or by experimental evidence (117) such as those in TarBase (118) (a manually curated database of experimentally supported miRNA targets). Understandably, an approach that aims to integrate these different computational algorithms and/or genome-wide miRNA/mRNA expression data such as ExprTarget (http://www.scandb.org/apps/microrna/) (105), therefore, could have the potential to generate a more reliable and comprehensive catalogue of the gene targets regulated by miRNAs, thus benefiting the studies on their role in other biological processes and physiological pathways. For instance, it is expected that pathway analysis on a more reliable and comprehensive list of gene targets of differentially expressed miRNAs between patient samples and normal controls could help construct a more precise model for the mechanisms of ALI susceptibility.

Since significant gene expression variation have been observed between human populations (60-63), studying the role of miRNAs in regulating population differences in gene expression would provide novel insights in health disparities such as the higher mortality rate in ALI (as well as sepsis) in African Americans and Hispanics in the United States (13). Although socioeconomic status could significantly affect health

disparities, notably, genes related to immune response to bacterial infection (e.g., genes in inflammatory pathways such as *CCR7*, chemokine receptor 7 and *CXCR3*, chemokine receptor 3) were found to be enriched among the differentially expressed genes between the cell lines derived from individuals of African and European ancestry (60, 119). Previous studies attempted to illustrate the contribution of SNPs and CNVs to population-level gene expression variation (60-63), similarly, it would be interesting to investigate the contribution of miRNAs to differential gene expression between populations as well, thus helping explain the observed racial difference in diseases including ALI. In addition, expression variation in some genes has also been observed between males and females using the cell line models (120, 121). Although genetics does not appear to affect gender-specific gene expression as males and females have the same autosomal genetic background, the contribution of miRNAs to gender-specific gene expression has not yet been studied. Therefore, it would be important to illustrate the role of miRNAs in defining gender-specific gene expression for the purpose of understanding the gender differences in inflammatory lung disease (e.g., gender differences in ARDS mortality rate (13)).

Because of the early stage of research, the current studies on the relationships between miRNAs and ALI/ARDS and VILI have been largely relied on animal models. No doubt, results derived from animal models can guide further investigations on patient samples and future translational research, the interpretation of these results, however, needs to be cautious as not all results from animal models are relevant to humans. Therefore, expanding the current studies to human cell lines, tissues and ultimately

human subjects would provide direct evidence to the role of miRNAs in the development of inflammatory lung disease.

Since miRNA expression is believed to be a dynamic process in cells, future experimental techniques that may monitor the longitudinal changes of miRNAs *in vitro* or *in vivo* and their interactions with changing cellular environment could provide unprecedented picture of the critical role of miRNAs in gene regulation and disease development. Finally, to construct a most comprehensive model for complex diseases such as ALI presents the challenges for integrating all kinds of data on the phenotypes or traits (e.g., gene expression, SNPs, CNVs, DNA methylation). MiRNAs will be critical components in our complete understanding of the mechanism and genetic networks of inflammatory lung disease. Though with some promising evidence so far, before they can be applied in the daily management of ALI, the potential of miRNAs to be novel therapeutic targets as well as biomarkers for ALI should also be continuously investigated.

Conclusion

Through their intimate role in gene regulation, miRNAs are emerging to be critical in understanding the underlying gene networks of complex diseases as well as phenotypes such as individual response to therapeutic treatments (15). Although at an earlier stage of investigation in ALI/ARDS and VILI relative to other common diseases including cancer, promising evidence on miRNAs' potentially critical role in disease susceptibility and progress of inflammatory lung disease has accumulated rapidly particularly in animal models. More basic research studies on miRNAs as well as the

gene targets regulated by these small RNA molecules are necessary. Further studies on the genetic variation related to miRNAs in real patient populations could benefit the ultimate goal of personalized medical care for inflammatory lung disease. It is expected that studies on miRNAs will facilitate the construction of a comprehensive disease model for ALI/ARDS or VILI by applying a systems biology approach that aims to integrate genetic, genomic, epigenetic, and proteomic as well as environmental information.

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References

1. Tomlinson L, Bellingan GJ. Trauma and acute lung injury. Trauma 2002;4(3):147-57.

2. Tsushima K, King LS, Aggarwal NR, De Gorordo A, D'Alessio FR, Kubo K. Acute lung injury review. Intern Med 2009;48(9):621-30.

3. Rubenfeld GD, Caldwell E, Peabody E, et al. Incidence and outcomes of acute lung injury. N Engl J Med 2005;353(16):1685-93.

4. Bream-Rouwenhorst HR, Beltz EA, Ross MB, Moores KG. Recent developments in the management of acute respiratory distress syndrome in adults. Am J Health Syst Pharm 2008;65(1):29-36.

5. Gajic O, Dara SI, Mendez JL, et al. Ventilator-associated lung injury in patients without acute lung injury at the onset of mechanical ventilation. Crit Care Med 2004;32(9):1817-24.

6. Dhanireddy S, Altemeier WA, Matute-Bello G, et al. Mechanical ventilation induces inflammation, lung injury, and extra-pulmonary organ dysfunction in experimental pneumonia. Lab Invest 2006;86(8):790.

7. Altemeier WA, Matute-Bello G, Frevert CW, et al. Mechanical ventilation with moderate tidal volumes synergistically increases lung cytokine response to systemic endotoxin. Am J Physiol Lung Cell Mol Physiol 2004;287(3):L533-42.

8. Bregeon F, Delpierre S, Chetaille B, et al. Mechanical ventilation affects lung function and cytokine production in an experimental model of endotoxemia. Anesthesiology 2005;102(2):331-9.

9. Wurfel MM. Microarray-based analysis of ventilator-induced lung injury. Proc Am Thorac Soc 2007;4(1):77-84.

10. Ware LB, Matthay MA. The acute respiratory distress syndrome. N Engl J Med 2000;342(18):1334-49.

11. Meyer NJ, Garcia JG. Wading into the genomic pool to unravel acute lung injury genetics. Proc Am Thorac Soc 2007;4(1):69-76.

12. Gong MN, Thompson BT, Williams P, Pothier L, Boyce PD, Christiani DC. Clinical predictors of and mortality in acute respiratory distress syndrome: potential role of red cell transfusion. Crit Care Med 2005;33(6):1191-8.

13. Moss M, Mannino DM. Race and gender differences in acute respiratory distress syndrome deaths in the United States: an analysis of multiple-cause mortality data (1979-1996). Crit Care Med 2002;30(8):1679-85.

14. Lim LP, Lau NC, Garrett-Engele P, et al. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 2005;433(7027):769-73.

15. Zhang W, Dolan ME. Emerging role of microRNAs in drug response Curr Opin Mol Ther 2010;12(6):695-702.

16. Reddy AJ, Kleeberger SR. Genetic polymorphisms associated with acute lung injury. Pharmacogenomics 2009;10(9):1527-39.

17. Kuba K, Imai Y, Rao S, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nature medicine 2005;11(8):875-9.

18. Imai Y, Kuba K, Rao S, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature 2005;436(7047):112-6.

19. Arcaroli JJ, Hokanson JE, Abraham E, et al. Extracellular superoxide dismutase haplotypes are associated with acute lung injury and mortality. Am J Respir Crit Care Med 2009;179(2):105-12.

20. Meyer NJ, Christie JD. Extracellular superoxide dismutase haplotypes and acute lung injury: reading into the genome to understand mortality? Am J Respir Crit Care Med 2009;179(2):89-91.

21. Bersten AD, Hunt T, Nicholas TE, Doyle IR. Elevated plasma surfactant protein-B predicts development of acute respiratory distress syndrome in patients with acute respiratory failure. Am J Respir Crit Care Med 2001;164(4):648-52.

22. Mora R, Arold S, Marzan Y, Suki B, Ingenito EP. Determinants of surfactant function in acute lung injury and early recovery. Am J Physiol Lung Cell Mol Physiol 2000;279(2):L342-9.

23. Christie JD. Interleukin-10, age and acute lung injury genetics: the action is in the interaction. Eur Respir J 2006;27(4):669-70.

24. Kono H, Fujii H, Hirai Y, et al. The Kupffer cell protects against acute lung injury in a rat peritonitis model: role of IL-10. Journal of leukocyte biology 2006;79(4):809-17.

25. Inoue G. Effect of interleukin-10 (IL-10) on experimental LPS-induced acute lung injury. J Infect Chemother 2000;6(1):51-60.

26. Abadie Y, Bregeon F, Papazian L, et al. Decreased VEGF concentration in lung tissue and vascular injury during ARDS. Eur Respir J 2005;25(1):139-46.

27. Medford AR, Ibrahim NB, Millar AB. Vascular endothelial growth factor receptor and coreceptor expression in human acute respiratory distress syndrome. Journal of critical care 2009;24(2):236-42.

28. Medford AR, Millar AB. Vascular endothelial growth factor (VEGF) in acute lung injury (ALI) and acute respiratory distress syndrome (ARDS): paradox or paradigm? Thorax 2006;61(7):621-6.

29. Tsangaris I, Tsantes A, Bonovas S, et al. The impact of the PAI-1 4G/5G polymorphism on the outcome of patients with ALI/ARDS. Thromb Res 2009;123(6):832-6.

30. Liu A, Mosher DF, Murphy-Ullrich JE, Goldblum SE. The counteradhesive proteins, thrombospondin 1 and SPARC/osteonectin, open the tyrosine phosphorylation-responsive paracellular pathway in pulmonary vascular endothelia. Microvascular research 2009;77(1):13-20.

31. Dhainaut JF, Charpentier J, Chiche JD. Transforming growth factor-beta: a mediator of cell regulation in acute respiratory distress syndrome. Crit Care Med 2003;31(4 Suppl):S258-64.

32. Gao L, Flores C, Fan-Ma S, et al. Macrophage migration inhibitory factor in acute lung injury: expression, biomarker, and associations. Transl Res 2007;150(1):18-29.

33. Flores C, Ma SF, Maresso K, Wade MS, Villar J, Garcia JG. IL6 gene-wide haplotype is associated with susceptibility to acute lung injury. Transl Res 2008;152(1):11-7.

34. Grigoryev DN, Ma SF, Irizarry RA, Ye SQ, Quackenbush J, Garcia JG. Orthologous gene-expression profiling in multi-species models: search for candidate genes. Genome Biol 2004;5(5):R34.

35. Flores C, Perez-Mendez L, Maca-Meyer N, et al. A common haplotype of the LBP gene predisposes to severe sepsis. Crit Care Med 2009;37(10):2759-66.

36. Christie JD, Ma SF, Aplenc R, et al. Variation in the myosin light chain kinase gene is associated with development of acute lung injury after major trauma. Crit Care Med 2008;36(10):2794-800.

37. Christie JD, Ma SF, Aplenc R, et al. Variation in the MYLK gene is associated with development of acute lung injury after major trauma. Crit Care Med 2008.

38. Flores C, Ma SF, Maresso K, Ober C, Garcia JG. A variant of the myosin light chain kinase gene is associated with severe asthma in African Americans. Genetic epidemiology 2007;31(4):296-305.

39. Gao L, Grant A, Halder I, et al. Novel polymorphisms in the myosin light chain kinase gene confer risk for acute lung injury. American journal of respiratory cell and molecular biology 2006;34(4):487-95.

40. Hong SB, Huang Y, Moreno-Vinasco L, et al. Essential role of pre-B-cell colony enhancing factor in ventilator-induced lung injury. Am J Respir Crit Care Med 2008;178(6):605-17.

41. Ye SQ, Simon BA, Maloney JP, et al. Pre-B-cell colony-enhancing factor as a potential novel biomarker in acute lung injury. Am J Respir Crit Care Med 2005;171(4):361-70.

42. Meyer NJ, Huang Y, Singleton PA, et al. GADD45a is a novel candidate gene in inflammatory lung injury via influences on Akt signaling. Faseb J 2009;23(5):1325-37.

43. Marshall RP, Webb S, Bellingan GJ, et al. Angiotensin converting enzyme insertion/deletion polymorphism is associated with susceptibility and outcome in acute respiratory distress syndrome. Am J Respir Crit Care Med 2002;166(5):646-50.

44. Wosten-van Asperen RM, Lutter R, Haitsma JJ, et al. ACE mediates ventilator-induced lung injury in rats via angiotensin II but not bradykinin. Eur Respir J 2008;31(2):363-71.

45. Jiang JS, Wang LF, Chou HC, Chen CM. Angiotensin-converting enzyme inhibitor captopril attenuates ventilator-induced lung injury in rats. J Appl Physiol 2007;102(6):2098-103.

46. Chen CM, Chou HC, Wang LF, Lang YD. Captopril decreases plasminogen activator inhibitor-1 in rats with ventilator-induced lung injury. Crit Care Med 2008;36(6):1880-5.

47. Sapru A, Curley MA, Brady S, Matthay MA, Flori H. Elevated PAI-1 is associated with poor clinical outcomes in pediatric patients with acute lung injury. Intensive Care Med 2010;36(1):157-63.

48. Prabhakaran P, Ware LB, White KE, Cross MT, Matthay MA, Olman MA. Elevated levels of plasminogen activator inhibitor-1 in pulmonary edema fluid are associated with mortality in acute lung injury. Am J Physiol Lung Cell Mol Physiol 2003;285(1):L20-8.

49. Garzon R, Calin GA, Croce CM. MicroRNAs in Cancer. Annu Rev Med 2009;60:167-79.

50. Zhang W, Dolan ME. Use of cell lines in the investigation of pharmacogenetic loci. Curr Pharm Des 2009;15(32):3782-95.

51. Simon BA, Easley RB, Grigoryev DN, et al. Microarray analysis of regional cellular responses to local mechanical stress in acute lung injury. Am J Physiol Lung Cell Mol Physiol 2006;291(5):L851-61.

52. Garcia JG. Searching for candidate genes in acute lung injury: SNPs, Chips and PBEF. Trans Am Clin Climatol Assoc 2005;116:205-19.

53. Zhang W, Ratain MJ, Dolan ME. The HapMap Resource is Providing New Insights into Ourselves and its Application to Pharmacogenomics. Bioinform Biol Insights 2008;2(1):15-23.

54. Duan S, Huang RS, Zhang W, et al. Genetic architecture of transcript-level variation in humans. Am J Hum Genet 2008;82(5):1101-13.

55. The International HapMap Consortium. The International HapMap Project. Nature 2003;426(6968):789-96.

56. The International HapMap Consortium. A haplotype map of the human genome. Nature 2005;437(7063):1299-320.

57. Cheung VG, Conlin LK, Weber TM, et al. Natural variation in human gene expression assessed in lymphoblastoid cells. Nat Genet 2003;33(3):422-5.

58. Morley M, Molony CM, Weber TM, et al. Genetic analysis of genome-wide variation in human gene expression. Nature 2004;430(7001):743-7.

59. Stranger BE, Forrest MS, Clark AG, et al. Genome-wide associations of gene expression variation in humans. PLoS Genet 2005;1(6):e78.

60. Zhang W, Duan S, Kistner EO, et al. Evaluation of genetic variation contributing to differences in gene expression between populations. Am J Hum Genet 2008;82(3):631-40.

61. Stranger BE, Forrest MS, Dunning M, et al. Relative impact of nucleotide and copy number variation on gene expression phenotypes. Science 2007;315(5813):848-53.

62. Stranger BE, Nica AC, Forrest MS, et al. Population genomics of human gene expression. Nat Genet 2007;39(10):1217-24.

63. Spielman RS, Bastone LA, Burdick JT, Morley M, Ewens WJ, Cheung VG. Common genetic variants account for differences in gene expression among ethnic groups. Nat Genet 2007;39(2):226-31.

64. Zhang W, Ratain MJ, Dolan ME. The HapMap Resource is Providing New Insights into Ourselves and its Application to Pharmacogenomics. Bioinform Biol Insights 2008;2:15-23.

65. Idaghdour Y, Storey JD, Jadallah SJ, Gibson G. A genome-wide gene expression signature of environmental geography in leukocytes of Moroccan Amazighs. PLoS Genet 2008;4(4):e1000052.

66. Zhang X, Richards EJ, Borevitz JO. Genetic and epigenetic dissection of cis regulatory variation. Curr Opin Plant Biol 2007;10(2):142-8.

67. Lau NC, Lim LP, Weinstein EG, Bartel DP. An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. Science 2001;294(5543):858-62.

68. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet 2004;5(7):522-31.

69. Lee CT, Risom T, Strauss WM. Evolutionary conservation of microRNA regulatory circuits: an examination of microRNA gene complexity and conserved microRNA-target interactions through metazoan phylogeny. DNA and cell biology 2007;26(4):209-18.

70. Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res 2006;34(Database issue):D140-4.

71. Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ. miRBase: tools for microRNA genomics. Nucleic Acids Res 2008;36(Database issue):D154-8.

72. Griffiths-Jones S. The microRNA Registry. Nucleic Acids Res 2004;32(Database issue):D109-11.

73. Bentwich I, Avniel A, Karov Y, et al. Identification of hundreds of conserved and nonconserved human microRNAs. Nat Genet 2005;37(7):766-70.

74. Enright AJ, John B, Gaul U, Tuschl T, Sander C, Marks DS. MicroRNA targets in Drosophila. Genome Biol 2003;5(1):R1.

75. He L, Gregory J H. MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet 2004;5(7):522-31.

76. Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 2009;19(1):92-105.

77. Garzon R, Calin GA, Croce CM. MicroRNAs in Cancer. Annu Rev Med 2009;60(1):167-79.

78. Gatt ME, Zhao JJ, Ebert MS, et al. MicroRNAs 15a/16-1 function as tumor suppressor genes in multiple myeloma. Blood.

79. Burchard J, Zhang C, Liu AM, et al. microRNA-122 as a regulator of mitochondrial metabolic gene network in hepatocellular carcinoma. Molecular systems biology 6:402.

80. Liu C, Yu J, Yu S, et al. MicroRNA-21 acts as an oncomir through multiple targets in human hepatocellular carcinoma. Journal of hepatology 53(1):98-107.

81. Hennessy E, O'Driscoll L. Molecular medicine of microRNAs: structure, function and implications for diabetes. Expert Rev Mol Med 2008;10:e24.

82. Lovis P, Roggli E, Laybutt DR, et al. Alterations in microRNA expression contribute to fatty acid-induced pancreatic beta-cell dysfunction. Diabetes 2008;57(10):2728-36.

83. Small EM, Frost RJ, Olson EN. MicroRNAs add a new dimension to cardiovascular disease. Circulation 121(8):1022-32.

84. Lu Y, Zhang Y, Shan H, et al. MicroRNA-1 downregulation by propranolol in a rat model of myocardial infarction: a new mechanism for ischaemic cardioprotection. Cardiovasc Res 2009;84(3):434-41.

85. Voellenkle C, van Rooij J, Cappuzzello C, et al. MicroRNA signatures in peripheral blood mononuclear cells of chronic heart failure patients. Physiol Genomics 42(3):420-6.

86. Matkovich SJ, Wang W, Tu Y, et al. MicroRNA-133a protects against myocardial fibrosis and modulates electrical repolarization without affecting hypertrophy in pressure-overloaded adult hearts. Circulation research 106(1):166-75.

87. Naga Prasad SV, Duan ZH, Gupta MK, et al. Unique microRNA profile in end-stage heart failure indicates alterations in specific cardiovascular signaling networks. J Biol Chem 2009;284(40):27487-99.

88. Mishra PJ, Mishra PJ, Banerjee D, Bertino JR. MiRSNPs or MiR-polymorphisms, new players in microRNA mediated regulation of the cell: Introducing microRNA pharmacogenomics. Cell Cycle 2008;7(7):853-8.

89. Mishra PJ, Song B, Mishra PJ, et al. MiR-24 tumor suppressor activity is regulated independent of p53 and through a target site polymorphism. PLoS One 2009;4(12):e8445.

90. Mishra PJ, Humeniuk R, Mishra PJ, Longo-Sorbello GS, Banerjee D, Bertino JR. A miR-24 microRNA binding-site polymorphism in dihydrofolate reductase gene leads to methotrexate resistance. Proc Natl Acad Sci U S A 2007;104(33):13513-8.

91. Duan S, Mi S, Zhang W, Dolan ME. Comprehensive analysis of the impact of SNPs and CNVs on human microRNAs and their regulatory genes. RNA Biol 2009;6(4):412-25.

92. Lindsay MA. microRNAs and the immune response. Trends Immunol 2008;29(7):343-51.

93. Wang S, Aurora AB, Johnson BA, et al. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev Cell 2008;15(2):261-71.

94. Moschos SA, Williams AE, Perry MM, Birrell MA, Belvisi MG, Lindsay MA. Expression profiling in vivo demonstrates rapid changes in lung microRNA levels following lipopolysaccharide-induced inflammation but not in the anti-inflammatory action of glucocorticoids. BMC Genomics 2007;8:240.

95. Pino-Yanes M, Tejera P, Corrales A, et al. Common variants of the interleukin-1 receptor-associated kinase 3 gene are associated with susceptibility to sepsis induced-acute lung injury. under review.

96. Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci U S A 2006;103(33):12481-6.

97. Nahid MA, Pauley KM, Satoh M, Chan EK. miR-146a is critical for endotoxin-induced tolerance: IMPLICATION IN INNATE IMMUNITY. J Biol Chem 2009;284(50):34590-9.

98. Bazzoni F, Rossato M, Fabbri M, et al. Induction and regulatory function of miR-9 in human monocytes and neutrophils exposed to proinflammatory signals. Proc Natl Acad Sci U S A 2009;106(13):5282-7.

99. Liu X, Zhan Z, Xu L, et al. MicroRNA-148/152 impair innate response and antigen presentation of TLR-triggered dendritic cells by targeting CaMKIIalpha. J Immunol 185(12):7244-51.

100. Liu G, Friggeri A, Yang Y, Park YJ, Tsuruta Y, Abraham E. miR-147, a microRNA that is induced upon Toll-like receptor stimulation, regulates murine macrophage inflammatory responses. Proc Natl Acad Sci U S A 2009;106(37):15819-24.

101. Tili E, Michaille JJ, Cimino A, et al. Modulation of miR-155 and miR-125b levels following lipopolysaccharide/TNF-alpha stimulation and their possible roles in regulating the response to endotoxin shock. J Immunol 2007;179(8):5082-9.

102. Wang X, Zhao Q, Matta R, et al. Inducible nitric-oxide synthase expression is regulated by mitogen-activated protein kinase phosphatase-1. J Biol Chem 2009;284(40):27123-34.

103. Sheedy FJ, Palsson-McDermott E, Hennessy EJ, et al. Negative regulation of TLR4 via targeting of the proinflammatory tumor suppressor PDCD4 by the microRNA miR-21. Nat Immunol 2010;11(2):141-7.

104. Ma F, Liu X, Li D, et al. MicroRNA-466l upregulates IL-10 expression in TLR-triggered macrophages by antagonizing RNA-binding protein tristetraprolin-mediated IL-10 mRNA degradation. J Immunol 184(11):6053-9.

105. Gamazon E, Im H-K, Duan S, et al. ExprTarget: An integrative approach to predicting human microRNA targets. PLoS ONE 2010;5(10):e13534.

106. Vaporidi K, Iliopoulos D, Francis RC, Bloch KD, Zapol WM. MicroRNA Expression Profile In A Murine Model Of Ventilator-induced Lung Injury. Am J Respir Crit Care Med 2010;181(1):A2031.

107. Zambon M, Vincent JL. Mortality rates for patients with acute lung injury/ARDS have decreased over time. Chest 2008;133(5):1120-7.

108. Spizzo R, Rushworth D, Guerrero M, Calin GA. RNA inhibition, microRNAs, and new therapeutic agents for cancer treatment. Clinical lymphoma & myeloma 2009;9 Suppl 3:S313-8.

109. Alevizos I, Illei GG. MicroRNAs as biomarkers in rheumatic diseases. Nature reviews 2010;6(7):391-8.

110. Dimmeler S, Zeiher AM. Circulating microRNAs: novel biomarkers for cardiovascular diseases? European heart journal. 2010;31(22):2705-7.

111. Ferracin M, Veronese A, Negrini M. Micromarkers: miRNAs in cancer diagnosis and prognosis. Expert Rev Mol Diagn 10(3):297-308.

112. Wang JF, Yu ML, Yu G, et al. Serum miR-146a and miR-223 as potential new biomarkers for sepsis. Biochemical and biophysical research communications 2010;394(1):184-8.

113. Vasilescu C, Rossi S, Shimizu M, et al. MicroRNA fingerprints identify miR-150 as a plasma prognostic marker in patients with sepsis. PLoS One 2009;4(10):e7405.

114. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 2005;120(1):15-20.

115. Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. Cell 2003;115(7):787-98.

116. Krek A, Grun D, Poy MN, et al. Combinatorial microRNA target predictions. Nat Genet 2005;37(5):495-500.

117. Sethupathy P, Megraw M, Hatzigeorgiou AG. A guide through present computational approaches for the identification of mammalian microRNA targets. Nat Methods 2006;3(11):881-6.

118. Papadopoulos GL, Reczko M, Simossis VA, Sethupathy P, Hatzigeorgiou AG. The database of experimentally supported targets: a functional update of TarBase. Nucleic Acids Res 2009;37(Database issue):D155-8.

119. Storey JD, Madeoy J, Strout JL, Wurfel M, Ronald J, Akey JM. Gene-expression variation within and among human populations. Am J Hum Genet 2007;80(3):502-9.

120. Zhang W, Huang RS, Duan S, Dolan ME. Gene set enrichment analyses revealed differences in gene expression patterns between males and females. In Silico Biol 2009;9(3):55-63.

121. Zhang W, Bleibel WK, Roe CA, Cox NJ, Eileen Dolan M. Gender-specific differences in expression in human lymphoblastoid cell lines. Pharmacogenet Genomics 2007;17(6):447-50.

122. Ghosh B, Sharma A, Kumar M, Mabalirajan U, Agrawal A. Identification of microRNA Involved in IL−10 Expression and Its Implication in Asthma. Am J Respir Crit Care Med 2009;179:A2491.

123. Tan Z, Randall G, Fan J, et al. Allele-specific targeting of microRNAs to HLA-G and risk of asthma. Am J Hum Genet 2007;81(4):829-34.

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

125. 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. Am J Respir Crit Care Med 2009;180(8):713-9.

126. 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. Proc Natl Acad Sci U S A 2009;106(44):18704-9.

127. 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.

128. Mohamed JS, Lopez MA, Boriek AM. Mechanical stretch up-regulates microRNA-26a and induces human airway smooth muscle hypertrophy by suppressing glycogen synthase kinase-3beta. J Biol Chem 285(38):29336-47.

129. Polikepahad S, Knight JM, Naghavi AO, et al. Proinflammatory role for let-7 microRNAS in experimental asthma. J Biol Chem 285(39):30139-49.

130. Christenson SA, Campbell JD, Zeskind J, et al. MicroRNA as regulators of gene expression changes that occur with the progression of emphysema. Am J Respir Crit Care Med 2010;181:A2024.

131. Sato T, Liu X, Nelson A, et al. Reduced miR-146a increases prostaglandin E in chronic obstructive pulmonary disease fibroblasts. Am J Respir Crit Care Med 182(8):1020-9.

132. Oglesby IK, Bray IM, Chotirmall SH, et al. miR-126 is downregulated in cystic fibrosis airway epithelial cells and regulates TOM1 expression. J Immunol 184(4):1702-9.

133. Pandit KV, Corcoran D, Yousef H, et al. Inhibition and role of let-7d in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 182(2):220-9.

134. Pottier N, Maurin T, Chevalier B, et al. Identification of keratinocyte growth factor as a target of microRNA-155 in lung fibroblasts: implication in epithelial-mesenchymal interactions. PLoS One 2009;4(8):e6718.

135. Liu G, Friggeri A, Yang Y, et al. miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. The Journal of Experimental Medicine 207(8):1589-97.

Table 1. Recent reports on microRNAs implicated in certain inflammatory lung diseases.

Condition	microRNA	Validated Gene Targets[#]	Functional Involvement	Reference
Asthma	miR-106a	IL-10	asthmatic features including airway inflammation	(122)
	miR-148a, miR-148b, and miR-152	HLA-G	risk of asthma	(123)
	miR-21	IL-12	allergic airway inflammation	(124)
	miR-133a	RHOA	bronchial hyperresponsiveness	(125)
	miR-126	OBF.1/BOB.1	T-helper 2 responses	(126, 127)
	miR-26a	GSK-3ß	airway smooth muscle hypertrophy	(128)
	let-7 miRNA family	IL-13	production of allergic cytokines	(129)
Chronic Obstructive Pulmonary Disease	miR-181d	IFNG, COL16A1		
	miR-30c	PCDH20, PHTF2, ELFN2		
	miR-150	IER5		
	18*	PLCH2	progression of emphysema	(130)
	miR-146a	PGE2	persistent inflammation	(131)
Cystic Fibrosis	miR-126	TOM1	IL-1beta and TNF-alpha-induced signaling pathways	(132)
Idiopathic Pulmonary Fibrosis	miR-let7d	HMGA2	epithelial and mesenchymal transition	(133)
	miR-155	KGF	epithelial-mesenchymal interactions	(134)
	miR-21	SMAD7	pro-fibrogenic activity of TGF-beta1	(135)

COL16A1: collagen, type XVI, alpha 1; ELFN2: extracellular leucine-rich repeat and fibronectin type III domain containing 2; GSK-3ß: glycogen synthase kinase 3ß; HMGA2: high mobility group AT-hook 2; HLA-G: major histocompatibility complex, class I, G; IER5: immediate early response 5; IFNG: interferon, gamma; IL-10: interleukin 10; IL-12: interleukin 12; IL-13: interleukin 13; KGF: keratinocyte growth factor; OBF.1/BOB.1: Oct binding factor 1 or B-cell Oct binding protein 1; PCDH20: protocadherin 20; PGE2: prostaglandin E2; PHTF2: putative homeodomain transcription factor 2; PLCH2: phospholipase C, eta 2; RHOA: ras homolog gene family, member A; SMAD7: SMAD family, member 7; TOM1: target of myb1.

Figure Legends

Figure 1. **Integrating microRNAs into the current approaches to identifying ALI associated genes.** The candidate gene approach focuses on known or putative mechanisms such as immune responses and lung biological pathways. The genome-based approach does not require *a priori* knowledge and is therefore unbiased and more comprehensive. Both approaches seek to identify genetic variants or gene expression phenotypes that are associated with the observed phenotypic variation. Integrating miRNAs into these paradigms could help explain results using both approaches. GWAS: genome-wide association study between phenotype and genotype; eQTLs: expression quantitative trait loci, i.e., distant or local SNPs associated with gene expression; miRSNPs: SNPs related to miRNA biogenesis and function.