MicroRNA networks during pneumonia and acute lung injury

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Summary

MicroRNAs (miRNAs) are a group of ubiquitous and highly conserved small noncoding RNA molecules which affect gene expression by acting on mRNA at a post-transcriptional level. Their role is being increasingly elucidated as they have shown to act as fine regulators of immune response and inflammatory pathways. MiRNAs also gained great interest from a clinical standpoint, as they could serve as both tissue-specific, early circulating biomarkers of disease and outcome predictors. Furthermore, they could represent a novel therapeutic strategy given that targeted administration or inhibition of miRNAs may result in a highly specific and effective treatment. However, poor knowledge exists about the role of miRNAs and their potential therapeutic use in respiratory infections and acute lung injury. In the present review we will discuss the current evidence regarding the application of miRNAs and their related technologies to these conditions.

KEY WORDS: microRNA, pneumonia, acute lung injury.

Introduction

Pneumonia is one of the leading causes of morbidity and mortality worldwide despite the availability of antimicrobial agents and preventive strategies. Lower respiratory tract infections represent the fourth cause of death globally, accounting for 41.6 deaths per 100,000, with an overall incidence of 291,759,000 million cases; together with influenza, they caused over 3.5 million deaths (6.1% of all death causes) in 2015 (1, 2). In Western Countries, deaths for both pneumonia and influenza range from 2.2 to 31.9 per 100,000 inhabitants per year (3, 4). Healthcare costs are high too: about 1.2 and 1.9 million hospital discharges respectively in United States and Europe (5, 6) with an estimated yearly burden-cost of over 10 billion dollars (7). Total costs including disability-adjusted life years (DALYs) have been estimated to be as high as 50 billion US dollars (8). Although several antibiotics and antiviral agents are available for most bacterial and influenza infections, there is an increasing concern for the occurrence of resistant strains to these drugs (9, 10). Moreover, survival may be further reduced if septic shock, acute respiratory distress syndrome (ARDS) and multi-organ failure syndrome (MOFS) occur as complications (11). Novel strategies are increasingly needed for the advanced management of pneumonia; these should be targeted to the immune-regulatory mechanisms of respiratory infections and should early detect potential complications. New genomic approaches have been employed to find biomarkers and therapeutic targets. Hereby, we will try to describe the role that micro-RNAs network may play in this regard.

MicroRNAs: molecular structure and biological function

MicroRNAs (miRNAs) are small noncoding double-stranded RNAs about 22 nucleotides pairs in length, with two free unmatched nucleotides at both 3' ends. At first described in animal models, they consist of small RNA molecules whose antisense region is complementary to protein-encoding mRNAs. As a result, they exert a repressive effect by reducing protein translation without altering the mRNA levels (12, 13). Currently, they are known to be involved in a number of regulatory pathways among diverse species (14-16). MicroRNAs are cleaved by an enzymatic complex (the Drosha RNase III endonuclease) in smaller hairpins of approximately 60-70 nucleotides and then transported from nucleus to cytoplasm (through Exportin-5), where they are once again elaborated by a large RNase III endonuclease called Dicer to produce their mature form. The RNA-induced silencing complex (RISC) is a ribonucleoprotein complex which incorporates microRNAs to allow them recognize their target complementary mRNA transcripts. A single microRNA may have a single or multiple mRNA targets, depending on the sequence (17, 18), but it may

also influence the expression of other miRNAs in a complex network (19). RISC can regulate gene expression in a number of ways including: mRNA degradation, translational repression, heterochromatin formation, DNA elimination. Alternatively, miRNAs can be synthetized from hairpin introns (miRtrons). These are transcribed, then spliced and de-branched by Lariat Debranching Enzyme (Ldbr) to form pre-miRNAs as Dicer substrates (20). A great number of miRNAs have been identified through computational genome analysis in animal and in human cells: a lot of them is highly conserved among species (21, 22). It is now estimated that miRNAs represent from 1 to 5% of the expressed genes in animal cells (1,000 to 50,000 copies of miRNA per cell), thus being the most important post-transcriptional regulatory system in eukaryotic cells (23). The miRNA network has a pivotal regulatory role in several biological processes such as development, cell signaling, proliferation, differentiation and apoptosis, and they show a specific expression in different cells and tissues (24). Alterations in miRNAs processing, expression or activity have been linked to a wide range of diseases (25-27). Focusing on pulmonary disease, miRNAs have been found to be important in lung development and differentiation, inflammatory and immune response, as well as in the pathogenesis of other specific respiratory disorders. Finally, circulating miRNAs which can be bound either to exosomes (28, 29), vesicle-free lipoproteins (30), or RNA-binding proteins (31) raise increasing interest as disease-specific biomarkers or therapeutic agents in pneumonia and acute lung injury.

MicroRNA-based regulation of inflammation and response to infection

MiRNAs are deeply involved in the regulation of both innate and adaptive immune response, as well as in driving the inflammatory pattern response to infection. MicroRNAs are key regulators of the inflammatory process and their expression is retroactively influenced by mediators of the inflammation itself. In innate immunity, dendritic cells, macrophages and granulocytes are triggered upon the recognition of specific pathogen-associated molecular patterns (PAMPs), such as bacterial lipopolysaccharide (LPS) or viral RNA, through toll-like receptors (TLRs) (32). Activation of TLRs triggers the intracellular signaling which ultimately activates NF-kB, a pivotal mediator of inflammatory and immune response; in particular, MyD88 pathway determines the transcription of pro-inflammatory genes, whereas TRF-mediated pathway induces the expression of interferon (IFN) response, according to the initial stimulus (33). Activation of TLRs also induces the expression of some miRNAs, which have been reported to have several regulatory functions (34). For example, miR-155 downregulates the expression of negative regulators such as SHIP1 and SOCS1, thus activating AKT (35, 36) and promoting inflammation. As a confirm of its role, experimental depletion of miR-155 resulted in a significantly decreased immune response, whereas its overexpression caused a myeloproliferative pattern (37-39). Moreover, downregulation of SOCS1 through miR-155 has been shown to enhance the innate antiviral response via type I IFN signaling (40). Another microRNA, the miR-146a, has been found to exert negative regulatory functions by reducing the mRNA levels of three important proteins involved in the TLRs mediated pathway which activates NF-kB: TRAF6, IRAK1 and IRAK2. As a consequence, the levels of pro-inflammatory cytokines such as IL-6 and TNF-alpha and type I IFN production during viral infections (41, 42) are lowered. Notably, miR-146a is induced by NF-kB and this determines a negative feedback loop which reduces the inflammatory response over time. MiR-21 is induced by TLR-4-mediated activation of NF-kB and it targets the mRNA of PDCD4 protein, which in turn activates NF-kB through unknown mechanisms. Downregulation of NF-kB and increased production of anti-inflammatory cytokines such as IL-10 are observed (43) accordingly. Other miRNAs involved in innate immune response are miR-125b and let-7, which target TNF-alpha and IL-6 mRNAs respectively, inhibiting them during acute inflammatory response (44, 45). Several miRNAs have been implied in the development and function of T cells. MiR-181a, for example, showed a regulatory role in the affinity selection of TCRs (T cell receptors) during T cells development in thymus. This by reducing the strength of intracellular TCR signaling in response to endogenous peptides, thus preventing the development of self-reactive T cells (46, 47). Also, miR-17 and miR-92 have been showed to enhance T cell survival through the inhibition of pro-apoptotic mechanisms mediated by Bcl-2-like protein 11 (BIM) and phosphatase and tensin homolog (PTEN) proteins. This may represent an important selection mechanisms of specific clones of T cells, but nonetheless it has been shown to favor lymphoproliferative diseases in animal models (48). During T cells clonal expansion, which arises in response to antigen recognition, miR-182 and miR-214 showed specific effects in selectively hindering the expression of anti-proliferative factors (49, 50). Other miRNAs, such as miR-155 and miR-327, play a role in influencing Th1 or Th2 differentiation of CD4+ T cells which is linked to cell mediated immune response and chronic inflammatory diseases (51, 52). InTable 1 a non-comprehensive list of miRNAs involved in regulation of immune response is reported.

MiRNAs as diagnostic and prognostic biomarkers in respiratory infections

Detection and characterization of circulating miRNAs in biological fluids is appealing as it would represent a minimally invasive tool for diagnosis, assessment of severity, prediction/monitoring of response to specific treatments and prognosis. Specific patterns of circulating miRNAs have been first identified in the field of cancer research (65, 66). Several single or panels of miRNAs have been reported in association with non-small cells and small-cells lung cancer (67). There is also ongoing research aimed at the identification of circulating miRNAs involved in non-neoplastic pulmonary disease, such as airways diseases (COPD and asthma) (68, 69),

Table 1 - Examples of miRNAs involved in immune response and host-pathogen interaction (see references for details).

miRNA	Functional status	Target	Effect (reference)
miR-155	Downregulated Orthologue mimicry	SHIP1 and SOCS1 (NF-κB pathway); Interaction with human rhinovirus RNA; B-cells regulatory pathways	Reduced adaptive immune response (reduction in IgM production and T cells stimulating cytokines) (53); Facilitation of rhinovirus respiratory infections (54); Kaposi's sarcoma herpes virus-induced B cells transformation (55)
miR-223	Downregulated	MEF2C (myeloid cells development)	Increased inflammatory response, increased susceptibility to acute lung injury (56)
miR-29 miR-146a*	Downregulated	INF-γ	Reduced T-cells and NK cells production of INF-γ upon mycobacterial infection (57, 58)
miR-146b* miR-1224	Upregulated	NF-κB pathway	Mycobacterial infection (58)
let-7e miR-21 miR-155 miR-210 miR-223	Upregulated	NF-κB pathway	Mycobacterial infection (virulent strains only) (58)
miR-122	Upregulated	Interaction with viral genome	Facilitation of hepatitis virus C infection (59)
miR-221	Downregulated	NGF/TRKA	Increased survival of airways epithelial cells upon RSV infection (60)
miR-132	Upregulated	p300	Facilitation of herpes virus family infections (61)
miR-128	Downregulated	Interaction with viral RNA	Facilitation of rhinovirus respiratory infections (62)
HIV-1 derived miRNA (TAR) miR-17/92 cluster	Upregulated Downregulated	ERCC1 and IER3 (apoptotic pathways) PCAF (histone acetyl-transferase)	Enhancement of HIV-1 replication (63, 64)

pulmonary fibrosis (70, 71), pulmonary hypertension (72-74), acute lung injury and pulmonary infectious disease. A wide range of miRNAs have been found potentially correlated with tuberculosis (75-77), whereas data

about pneumonia and other respiratory infections are still poor. One study from Abd-El-Fattah et al. (78) evaluated 148 patients admitted to the hospital for pleural effusion which underwent diagnostic thoracentesis: their serum miRNAs levels were measured and matched against 37 healthy controls. MiR-21, miR-155, miR-182, and miR-197 levels were found to be significantly increased in the affected subjects when compared to controls, with different profiles according to the etiology of pleural effusion. In particular, miR-155 showed a greater diagnostic accuracy for para-pneumonic pleural effusions (AUC 1.0, 95% CI 1.0-1.0, P <0.0001), whereas miR-21 and miR-197 were increased to a lesser extent. In patients with active tuberculosis only miR-197 was increased whereas mir-182 showed the best correlation with neoplastic pleural effusions, followed by miR-155. It must be noted though that the design of this study, namely the high case-to-control ratio, hamper its clinical significance. Few studies in animal models and humans are available about viral influenza. Vela et al. (79) evaluated the pattern of miRNA expression in mice upon infection with different influenza viruses: seasonal H1N1 (A/Texas/36/91), swine H1N1 (A/California/04/09) and the highly pathogenic H5N1 (A/Vietnam/1203/04). Animals underwent a complete histopathologic examination and assessment of systemic involvement, whereas miRNAs quantitative analysis took place on lung tissues at 6, 12, 24, 72 and 96 h after viral challenge. In particular, miR-49, miR-301a and miR-141 were found to be overexpressed early after viral challenge. Notably, infection with A/Vietnam/1203/04 led to greater changes in a higher number of specific miRNAs. MiR-301a is linked to the host immune response by activating the NF-κB signaling, whereas miR-141 has been implicated in facilitating infection and propagation of several viruses, including H5N1. Also, miR-200a, miR-223 and miR-21 appeared to be variably upregulated after challenge with all three viral strains, thus suggesting a role of these miRNAs in facilitating viral infections through increased viral pathogenicity and reduced expression of antiviral cytokines such as TNF-α. Another recent study (80) evaluated the expression of miRNAs in mice infected with the A/Puerto Rico/8/34 H1N1 influenza virus. Survival rate, weight loss and histopathologic modification were evaluated along with mRNA and miRNA profiling from lung tissue. A total of 82 miRNAs and 3371 mRNAs were expressed differently after infection. Using bioinformatics algorithms, 17 miRNAs were matched to the regulation of influenza A infection pathway, including miR-21, miR-7a, miR-449a, and miR-34. From a clinical standpoint, Moran et al. (81) evaluated the profile of circulating miRNAs in subjects infected with pandemic H1N1 influenza virus. An equal number of subjects with severe and mild A/H1N1 induced pneumonia and asymptomatic contacts, plus 5 healthy controls (29 subjects in total) were evaluated. In patients with severe pneumonia, eight miRNAs (miR-374a, miR-875-5p, miR-342-3p, miR-150, miR-15b, miR-376a, miR-376c, miR-214) were significantly upregulated, which through bioinformatics analysis were found to target at TGF-β, apoptosis and Wnt/β-catenin related pathways. Also, down-regulation of miR-29c, miR-1247, miR-1233 was reported in severe H1N1 pneumonia as compared to the other groups; moreover, patients with mild H1N1 pneumonia showed a characteristic pattern of increased expression of miR-601, miR-520c-3p, miR-1183, miR-1303 and miR-21. Additionally, recent data show that specific miRNA profiles affect the production of pro-inflammatory mediators, suggesting the potential role of miRNAs as biomarkers of disease progression in pneumonia (82, 83). Moran et al. (84) documented increased levels of miR-150 in the peripheral blood of critically ill patients with H1N1 infection compared to people with milder disease and healthy controls. In addition, they found downregulation of miR-22 in patients with severe A/H1N1 disease. In previous studies, miR-22 has been shown to be involved in cell cycle and apoptosis (85). Other studies have underlined the role of another miRNA family in the prognosis of A/H1N1 infection, the miR-29 (86), which participates in immune regulation inhibiting IFN-γ production from T cells (87).

In the field of acute lung injury and acute respiratory distress syndrome (ARDS), the role of miRNAs as diagnostic and prognostic (135) biomarkers has been reviewed recently. Sun et al. (88) examined the role of miR-181b (which is involved in the regulation of inflammatory response) in patients with sepsis or sepsis plus ARDS compared with control subjects admitted to the ICU without sepsis. MiR181b is an inhibitor of the NF-κB pathway: it targets the importin-α3, which in turn inhibits nuclear accumulation of p50 and p65, thus reducing the expression of adhesion molecules, leukocyte accumulation, and vascular inflammation (120). Circulating levels of miR-181 were found to be reduced by approximately 40% in patients *versus* controls. The Authors also proved that rescue of miR-181b levels reduced lung injury and mortality in an animal model consisting of endotoxemic mice. Guo et al. (89) evaluated the role of miR-125b, whose downregulation is known to be linked to the development of ALI, in a double armed murine and human study. In a murine model of LPS-induced ALI, they showed that up-regulation of miR-125b maintained the body weight, resulted in better survival and significantly reduced LPS-induced pulmonary inflammation and vascular permeability, inducing overall amelioration of histopathologic findings. In the human arm of the study, 16 subjects with moderate to severe ARDS (matched towards healthy controls) showed a significant reduction of miR-125b levels, which were inversely correlated with circulating levels of interleukin-6 and TNF-α, as well as with indicators of disease severity and prognosis such as PaO2/FiO2 ratio, Acute Physiology and Chronic Health Evaluation (APACHE) II, Simplified Acute Physiology Score (SAPS) II and the Murray Lung Injury Score (LIS). A similar study from Yang et al. (90) evaluated 45 patients who developed ALI within 24 hours from cardiopulmonary bypass (CPB). Arterial blood samples were collected at 5 different time point: prior to CPB (T0) to 16 h after reperfusion (T4). At T4, compared with T0 time point, 11 miRNAs levels were significantly altered, in particular miR-499 (0.25 folds reduction) and in miR-320 (3.56 folds increase). The blood tumor necrosis factor-α (TNF-α) level and patient's respiration index (RI) positively correlated with the miR-320

level, whereas the PaO2/FiO2 ratio was negatively associated with miR-320. Furthermore, an *in vitro* model of reperfusion injury based on human alveolar type II epithelial A549 cells showed that miR-320 was significantly increased after injury. Altogether, data from these studies suggest that the development of automated miRNA panels testing may play a crucial role in the clinical practice as a tool for early diagnosis, pathogen identification, quantification of disease severity and development of novel therapies targeted at specific miRNA-regulated pathophysiologic mechanisms. Table 2 depicts a summary of potential biomarkers in respiratory infections and lung injury.

Is there a role for microRNAs as therapeutic targets or agents in pneumonia and ALI?

Finally, we will review the most recent advances concerning miRNAs as therapeutic targets or agents in experimental settings. Early studies showed that stimulation of macrophages with agonists that activated TLR2, TLR3, TLR4, or TLR9 resulted in increased expression of miRNAs (91). The coupling of these pathways may provide critical regulatory signals that activate innate host defense and subsequently induce inflammation necessary for pathogen clearance (92, 93). Accordingly, delivery of LPS (which signals via TLR4) to the airways

Table 2 - Examples of miRNAs involved as biomarkers in pulmonary infections and ALI/ARDS (see text and references for details).

miRNA	Functional status	Location	Disease
miR-29a, let-7e, miR-146a, miR-148a, miR-16, miR-192, miR-193a-5p, miR-25, miR-365, miR-451, miR-532-5p, miR-590-5p, miR-660, miR-885-5p, miR-223*, miR-30e, miR-93;	Upregulated	Circulation	Active Tuberculosis
miR-197	Upregulated	Circulation	Tubercular pleural effusion
miR-155, miR-21, miR-197	Upregulated	Circulation	Parapneumonic pleural effusion
miR-49, miR-301a, miR-141, miR-200a, miR-223 and miR-21, miR-7a, miR-449a, miR-34;	Upregulated	Lung tissue	Influenza virus in mice (different strains)
miR-374a, miR-875-5p, miR-342-3p, miR-150, miR-15b, miR-376a, miR-376c, miR-214;	Upregulated	Circulation	Severe H1N1 influenzal pneumonia (in humans)
miR-29c, miR-1247, miR-1233;	Downregulated		
miR-601, miR-520c-3p, miR-1183, miR-1303 and miR-21.	Upregulated	Circulation	Mild H1N1 influenzal pneumonia (in humans)
miR-181b	Downregulated	Circulation	Sepsis or Sepsis+ARDS (humans)
miR-125b	Downregulated	Circulation, lung tissue	Development of ALI/ARDS (both animal models and humans)
miR-499	Downregulated	Circulation, epithelial alveolar cells (*in vitro*)	
miR-320	Upregulated		Development of ARDS after cardiopulmonary bypass

of mice resulted in increased expression of miR-21, miR-25, miR-27a, miR-100, miR-142-3p, miR-146a, miR-181c, miR-187, miR-199 and miR-223, correlated with neutrophilic recruitment (94). Subsequent studies using mice deficient in a specific miRNA have demonstrated that a single miRNA can lead to the development of pulmonary inflammation (95-97) but it can also have anti-inflammatory effects under other circumstances (98, 99). MiR-155, for instance, plays a pivotal role in the proinflammatory activities of macrophages, monocytes, and dendritic cells (DCs) (95, 100-109). When mouse macrophages (classically activated or M1 phenotype) or human monocytes are stimulated with LPS, an increased expression of miR-155 results, which is linked to the production of tumor necrosis factor-a (TNF-α) and antiviral responses through the activation of IFN-β signaling pathways (92, 99, 110). Increased expression of miR-155 in antigen-presenting cells after exposure to various inflammatory mediators appears to be induced by the proinflammatory nuclear factor enhancer of activated B cells (NF-kB) and Jnk signaling pathways (18-20). Moreover, IL-10 exerts its anti-inflammatory effects on macrophages and monocytes by inhibiting the expression of miR-155 (94, 108) and IL-13 plays an important role in the polarization of macrophages to the M2 phenotype via IL13Rα1, a component of IL-4R which is targeted by miR-155 (111). Rodriguez et al. (98) gave one of the first demonstrations that mice deficient in bic/miR-155 are immunodeficient and show increased remodelling of airway tissue, associated with a higher number of Th2 and eosinophils in bronchoalveolar lavage fluid. The same nucleic acid regulates not only the innate immune response, but also adaptive and humoral ones since activation of B and T lymphocytes results in increased miR-155 expression: this is confirmed by studies in which miR-155 deficient mice have abnormal humoral responses to infection and allergens (110-113) and die after 7 days from oral challenge with s. typhimurium (98), thus showing a less efficient protection by the vaccination itself. Verschoor et al. (114) gave a further evidence of the role of miR-155 in S. Pneumoniae clearance from the nasopharynx. Interestingly, this does not affect pneumococcal invasion and survival rate to 5-days post inoculation of the high virulence P1547 (serotype 6A) strain but mir155KO mice were significantly impaired in their ability to clear a less virulent colonizing strain (113). On the contrary, Podsiad et al. (115) hypothesized that upregulation of miR-155 inhibits IL-17 and increases susceptibility to secondary bacterial pneumonia after viral infection. Mice were challenged with H1N1 intra-nasally and then infected with methicillin-resistant Staphylococcus aureus (MRSA) intra-tracheally after 5 days. An antisense oligonucleotide (antagomiR-155) improved bacterial clearance by 4.2 fold compared with control antagomiR in postsequential infection with virus and bacteria. Recently, miR-155 targeting has also been proposed in Staphylococcal enterotoxin B (SEB) inhalation, which can trigger acute inflammatory lung injury via immune cell infiltration and excessive cytokine production (116). MiR-155-deficient mice were protected from SEB-mediated inflammation and acute lung injury, thereby suggesting that therapeutic target. The high levels of IFN-γ production associated with SEB exposure can be attributed to the miR-155-mediated repression of *Socs1*, a critical regulator of IFN-γ.

Apart from inflammatory injury during infection, a role for microRNAs in the regulation of acute lung injury has been identified quite recently. The study from Worm et al. (117) was the first to describe the effect of miR-155 ASO (antisense oligonucleotide) treatment on the recovery of ALI. Similarly, more recent research work reported that silencing miR-21 could affect the development of HVTV-induced murine lung injury (118), significantly increasing lung neutrophils and the keratinocyte-derived chemokine. Recruitment and expansion of Tregs are critical factors for recovery from ALI (119). In fact, enhanced expansion of Tregs *in vivo*, which is dominantly induced by IL-10-secreting M2-like macrophages, is critical for their elevated proportion in miR-155 ASO-treated ALI mice. Based on this evidence, the study from Guo et al. (120), provides C/EBPβ (a target molecule of miR-155 which is upregulated and associated with IL-10 secretion and M2-like phenotype of macrophages) as a previously unknown mechanism for mi-RNA based therapy against ALI. MiR-146 is another non-coding RNA whose expression has been well-related to LPS exposure and ALI (121). It acts as an NF-κB-dependent inhibitor which targets the signaling proteins of innate immune response to LPS. MiR-146a controls TLRs and cytokine signaling through a negative feedback loop involving down-regulation of IL-1 receptor-associated kinase 1 and TNF receptor-associated factor 6 (122). Similarly to miR-146a, the expression of miR-147 appears critical for endotoxin-induced tolerance (108, 123). The anti-inflammatory effect of miR-146 upregulation in LPS-induced ALI model (124), suggests it may be therapeutically targeted as a mean to repress inflammatory response after ALI. The importance of miR-146 in repressing the TLR-2 downstream mediators after pneumococcal infections has been recently confirmed (125). A study from Gauna and Cha (126), however, found that miR-146 overexpression protects against aseptic ALI (e.g. following gastric acid aspiration) but not against pneumonia- or sepsis-related ARDS. Further examination of the miR-127/CD64 axis may also provide new insights into the regulation of ALI (91, 121). In fact, intratracheal administration of miR-127 resulted in an exaggerated pulmonary inflammation and injury (127). Conversely, antagonization of miR-127 suppressed the production of proinflammatory cytokines and rendered the mice more refractory to inflammation-associated pathologies by blocking JNK-mediated M1 differentiation, but also boosting the expression of the anti-inflammatory cytokine IL-10 and M2-featured genes (128). Other miRNA molecules take part in the inflammatory response in pneumonia and/or ALI (124, 127, 135-137). However, very little is known on their potential therapeutic application. MiR-135a and miR-181, for example, seem to have a role in LPS-induced apoptosis of A549 cells by targeting Bcl-2 (129). Two main studies (129, 130) suggest that inhibition of these small RNAs might serve in the prevention of sepsis- or LPS-related ALI. Furthermore, miR-181b was shown to act as a proinflammatory factor

targeting the NF-κB signaling pathway *in vitro* (131). With regards to these molecules, more research is needed in order to precisely define their behaviour *in vivo* as well as their potential therapeutic implications. As a last one to count, miR-454 targeted at the 3'-UTR of CXCL12 mRNA inhibits its protein translation in human lung epithelial cells *in vitro* (132, 133). Similarly, overexpression of miR-454 in mice lungs significantly reduced the LPS-induced permeability and production of inflammatory cytokines CXCL1, CXCL2, IL6 and TNFα, thus suggesting a promising therapeutic approach to reduce the severity of ALI (122). Martelli et al. (134) generated lentiviral vectors which express both microRNA and antisense-RNAs targeting the 5' end of the PA, PB1 and PB2 influenza virus genomic sequences. The expression of RNA molecules led to a decreased viral load when transducted A549 cells were challenged with different human and avian influenza A virus strains, whereas no inhibition of influenza type B was observed (135). This supports the evidence that targeting specific regions of the influenza genomic sequences might represent an efficient strategy to inhibit viral replication. Even if it is clear that miRNAs offer potential new elements to treat diseases of the immune system or improve host defence, few concerns exist when it comes to practice hence the employment of direct inhibitors of miRNA pathways (e.g. antagoMIRs) needs to be approached with caution. For example, inappropriate manipulation of miRNA function may predispose to cancer, impaired immunity, or other cellular abnormalities (91). Furthermore, a single small RNA usually regulates multiple transcriptional targets: this is at the same time a great advantage and a major disadvantage due to potential side effects. As a consequence, a major issue becomes the delivery of miRNAs only to the diseased tissue but not to healthy ones in order to limit any disruption of their homeostasis (91).

The role of microRNAs in lung regeneration after pneumonia and acute lung injury

After pathogen-induced acute lung injury, often remodelling occurs, which eventually impairs lung function. For this reason, the repair stage of influenza pneumonia is gaining increasing interest. In this regard Tan et al. (137) profiled miRNA and mRNA expression levels following lung injury and tissue regeneration using a murine H1N1 influenza pneumonia model. Lungs were harvested at 7 and 15 days post-infection to evaluate the expression of selected miRNAs and mRNAs in those which displayed similar degrees of injury. Histopathological analyses of infected lungs showed more damaged areas and a greater number of infiltrating polymorphs at 7 days, whereas appearance of new epithelial cells was observed at day 15 as confirmed by immunohistochemistry. At 7 days post-infection, functions and pathways such as immune cell proliferation, inflammation, and innate viral responses were significantly enriched, while repair functions were relatively sparse. On the other hand, following viral clearance by 15 days post-infection, the pathway enrichment shifted from immune responses to largely cellular responses of repair and proliferation, such as DNA damage response, cellular proliferation, DNA replication and cell cycle activities. Compared to controls, infected mice showed 5 times more differentially expressed miRNAs, with almost no overlap between miRNA or mRNA expression at the two timepoints. The Authors narrowed their selection down to 3 miRNAs at 7 dpi (miR-290, miR-1940, miR-505) which were marked to be crucial in the early repair phase. At 15 dpi, 17 miRNAs (let-7b,c, miR-10a, miR-21, miR-25, miR-26a, miR-29c, miR-30a,b,c,d, miR-99a, miR-103, miR-151, miR-195, and miR-200b,c) were identified to be important in the late repair phase according to the literature. With the limit of small population samples, this study underlines the importance of miRNAs in lung regeneration following lung injury; however, it is still far from giving concrete therapeutic insights. Further studying is needed to better elucidate potential miRNAs that are crucial for lung repair and pulmonary regeneration.

Conclusions

The regulatory role of microRNA networks on the immune response and repair processes during pneumonia and acute lung injury is emerging from a number of experimental data, but information from human pathology are still lacking. A deeper knowledge of miRNAs interactions with immune response, the inflammatory cascade and their specific triggers could hopefully result in new anti-inflammatory therapies, as well as in strategies to improve pathogen clearance from the lung. Diverse circulating microRNAs has been suggested as useful diagnostic and prognostic biomarkers of pneumonia, but validation studies are needed before enabling their use in the clinical practice.

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