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Chapter 10

Emerging Roles of microRNAs in Cystic Fibrosis — From Pathogenesis to Development of New Therapies

Sabrina Noel and Teresinha Leal

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Abstract

As essential components of the regulatory system of gene expression, microRNAs (miRNAs) have been shown to influence development, severity, prognosis, and/or progression of a variety of inherited diseases. Differential expression studies have evidenced an impact of miRNAs on lung disease development in chronic obstructive pulmonary disease (COPD), asthma, lung inflammation, consequences of smoke exposure and airway allergy in human and in animal models of the diseases. Recent clinical and cell-based studies have revealed specific alterations of miRNA expression in cystic fibrosis (CF). Here we critically review the major findings concerning altered miRNA expression in CF airway epithelium, in particular with respect to CF transmembrane conductance regulator (CFTR) expression, innate immunity, and epithelial differentiation. Finally, we explore strategies to exploit these changes with the aim of innovative therapeutic benefits.

Keywords: microRNA, cystic fibrosis, CFTR, lung pathophysiology, inflammation, epithelial differentiation, biomarkers

1. Introduction

Cystic Fibrosis (CF) is due to mutations in the CF transmembrane conductance regulator (CFTR) gene causing impairment of chloride ions exchanges through the apical membrane of epithelial cells. CF affects epithelia in a variety of organs, notably lung, intestine, pancreas and the reproductive system. The most common CFTR mutation, F508del, results in deletion of a

phenylalanine at position 508 of the protein, and the mutated protein is retained in the endoplasmic reticulum and rapidly degraded via the endoplasmic reticulum-associated degradation pathway. Up to now, almost 2000 CFTR mutations have been identified (http://genet.sickkids.on.ca). A good correlation can generally be observed between *CFTR* genotype and the gastrointestinal disease, the pancreatic status, and the reproductive tract abnormalities, but the lung disease outcome is difficult to predict based solely on *CFTR* genotype (http://www.cftr2.org/). For example, it has been shown that siblings and monozygous twins, thus carrying the same *CFTR* genotype, even if living in the same environment and receiving the same medical care, may develop different spatial and temporal patterns in lung disease progression. The basis for variability in severity of CF lung disease is poorly understood and depends on concomitant expression of other genetic and environmental factors. Over the recent years, exploring the role of genetics/genomics (e.g. modifier genes, gene-environment interactions, epigenetics, etc.) has received growing attention in CF, with the aim of unveiling basic mechanisms and bringing in better understanding of the pathophysiology of the disease, helping to predict its progression, and hopefully leading to specially designed novel therapeutic strategies.

Of the whole human genome, only a small fraction, the protein-coding part, has long attracted attention because of the pervasive role of genes in determining amino acid sequences of expressed proteins, leading to observable consequences of mutations. Later on, following the discovery of transduction factors regulating gene translation, the initial view of "junk DNA", corresponding to the majority of DNA, had to be revisited. Following the identification of non-messenger RNA functions, the new concept of a network of non-coding transcriptome that regulates protein-coding expression emerged.

Non-coding RNAs are presently broadly categorized into three classes. The major class (well over 90% of total RNA) makes up the so-called housekeeping RNAs; they consist of small nuclear, small nucleolar, transfer, and ribosomal RNAs, the latter interacting with protein and transfer RNA to form the functional ribosome complex. The other classes of non-coding RNAs are long (>200) and short (<200) ribonucleotides. The best characterized and most extensively studied family of non-coding RNAs is that of microRNAs (miRNAs), short (17--27 nucleotides in length) single-stranded RNA molecules, which negatively regulate the translation of messenger RNAs into proteins. Figure 1 summarizes the general mechanism of biogenesis of miRNAs. Highly phylogenetically conserved, they bind to the 3'UTR (untranslated region) of target mRNAs, thereby potently repressing the target mRNA translation into protein or favoring mRNA degradation. To date, more than 1800 mature miRNAs have been identified in the human genome (http://www.mirbase.org). Bioinformatics studies predict that miRNAs potentially regulate the expression of about 60% of human genes.

As essential components of the regulatory system of gene expression, miRNAs have been shown to influence the development, the severity, the prognosis and/or the progression of a number of inherited diseases [1]. Differential expression studies of miRNAs have evidenced an impact on lung disease development in chronic obstructive pulmonary disease (COPD), asthma, lung inflammation, consequences of smoke exposure and airway allergy in human and in animal models of the diseases [2]. Since marked inflammation is a major feature of CF

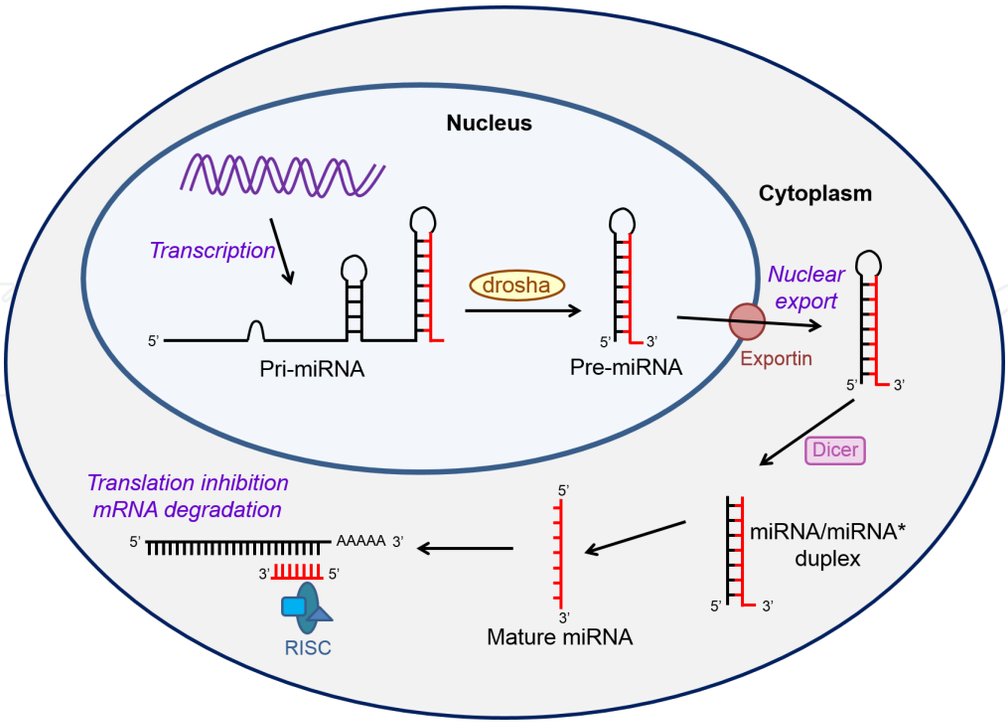

Figure 1. Biogenesis of microRNAs (miRNAs). miRNA genes are transcribed by RNA-polymerase 2 into primary miRNA (pri-miRNA) precursors. The pri-miRNAs are cleaved in the nucleus by a nuclear protein complex including the class 2 ribonuclease III Drosha to produce pre-miRNAs. Pre-miRNAs are exported to the cytoplasm via the nuclear export protein Exportin. In the cytoplasm, a pre-miRNA is cleaved by a protein complex including the helicase Dicer and form a duplex of miRNAs containing the mature miRNA bound to its complementary sequence (miRNA*). Duplexes are unwound and mature miRNAs bind to the 3'UTR of the target mRNA within the RNA-induced silencing complex to prevent translation by inhibition of ribosomes binding or mRNA degradation.

lung disease, miRNAs may be expected to play a role in its pathogenesis. Indeed, recent clinical and cell-based studies have revealed CF-specific alterations in miRNA expression. This point will be extensively discussed further below.

In this article, we review and highlight some of the most relevant published data focusing on miRNAs in CF. The first section deals with regulation of CFTR expression and modulation of CFTR trafficking. In the second section, key elements of inflammatory and innate immune responses in with CF airways are reviewed, focusing on the potential role of miRNAs in molecular pathways involved in lung inflammation. The third section discusses the potential role of miRNAs in lung development, differentiation and remodeling. The next section explores strategies to exploit miRNAs as biomarkers and potential therapies of CF disease. Finally, limitations in translating miRNAs from deeper knowledge of their role in pathogenesis to development of new therapies are highlighted.

2. Regulation of CFTR expression

The human *CFTR* gene is located on chromosome 7 and spans 189 kb. Its mRNA transcript is 6.2kb long and includes a 1.5kb 3'UTR containing multiple potential binding sites for miRNAs.

According to computational analyses using common bioinformatic programs such as TargetScan (http://www.targetscan.org), PicTar (http://pictar.mdc-berlin.de), and miRanda (http://www.microrna.org), CFTR 3'UTR contains almost 500 putative miRNA target sites, several of which have already been examined.

The main miRNAs involved in cystic fibrosis pathogenesis are summarized in figure 2.

Gillen et al. were the first to experimentally show that miRNAs could regulate CFTR expression [3]. Among the numerous miRNAs putatively repressing CFTR mRNA, twelve miRNAs (miR-145, miR-331-3p, miR-376a/b, miR-377, miR-384, miR-494, miR-600, miR-607, miR-939, miR-1246, miR-1290 and miR-1827) were able to decrease CFTR mRNA levels in the human Bronchial epithelial (HBE) 16HBE-14o-, colon carcinoma epithelial Caco-2 and the pancreatic adenocarcinoma PANC-1 cell lines. Direct targeting of the CFTR 3'UTR was evidenced for miR-145 and miR-494 in Caco-2 cells. The results [3] showed that the pattern of miRNAs effects on targeted CFTR 3'UTR varies with cell lines, suggesting that miRNA-driven gene expression could be tissue specific. Another study [4] demonstrated direct repression of CFTR expression by miR-101 and miR-494 in the HEK (human embryonic kidney) cell line expressing CFTR 3'UTR constructs. miR-101 and miR-494 seem to bind directly to the CFTR 3'UTR at positions 1508–1514 and 1140–1147, respectively. Downregulation of CFTR gene expression by miR-101 as well as by miR-144 and miR-145 was further confirmed in the 16HBE14o- cell line and in primary human airway cells, which are relevant models of CF lung disease [5, 6].

In a non-CF context, expression of miR-101 and miR-144 was previously shown to be upregulated in lungs upon exposure to air pollutants (such as cigarette smoke or cadmium) and correlated with loss of CFTR expression [4]. Hassan et al. [5] demonstrated upregulation of miR-101 in vivo in mice exposed to cigarette smoke, a condition associated with acquired loss of CFTR function [7]. miR-101 was also found highly expressed in lungs of COPD smoking patients in comparison with that of healthy non-smoking subjects. Moreover, miRNA expression profile analysis performed in the CF bronchial brushings in comparison to healthy controls showed high levels of miR-101, thus further reinforcing the potential role of miR-101 in CF [8]. Interestingly, synergistic effects between miR-101 and miR-494 were observed, although they are targeting different sites in CFTR 3'UTR [4]. Similar synergy was found for miR-509–3p and miR-494 [9]. Therefore, distinct miRNAs may act cooperatively to regulate CFTR expression and function in primary airway epithelial cells.

These miRNAs are likely able to modulate F508del-CFTR mRNA expression as well. Interestingly, increased expression of miR-145, miR-223 and miR-494 in vivo has been shown to correlate with decreased CFTR expression in bronchial epithelium of individuals bearing the F508del-CFTR mutation [3] as well as in CFBE41o- cells [10]. Experimental modulation of miRNA expression confirmed the hypothesis supporting the view that deregulation of miRNA may affect CFTR biogenesis in CF cells. Another study demonstrated that miRNA can indirectly influence CFTR biogenesis by modulating expression of other regulatory elements such as transcription factors [11]. It has been shown that modulating the transcription regulation factor SIN3A expression with miR-138 mimics increased biogenesis and cell surface expression of both wild-type and F508del-CFTR proteins. Interestingly, miR-138 indirectly

prevented proteosomal degradation of F508del-CFTR mutant and favored its trafficking towards the apical membrane in HBEs from CF patients [11].

Altogether, these data are in favor of a role for several miRNAs in the post-transcriptional regulation of the CFTR channel synthesis and trafficking. Because several of them are deregulated in CF, they could play a major role in CF lung pathology.

3. Regulation of key elements of inflammation and innate immune system

miRNAs are crucial regulators of innate and adaptive immune responses and their abnormal expression or function or both have been linked to multiple human inflammatory disorders [12]. The link between *CFTR* mutations and the mechanisms underlying disproportionate proinflammatory responses remains poorly understood. Aberrant release of proinflammatory mediators by epithelial and immune cells in CF includes hypersecretion of interleukin-8 (IL-8). The fact that this can be detected in lungs of children with CF [13] even during fetal life [14, 15] suggests that it is a constitutive process of CF. miRNA-based post-transcriptional regulations, known to be highly sensitive to a range of homeostasis signals, such as changes in hypoxia, pH, ion concentration and osmolarity, have recently been considered as potentially regulating immune responses in CF, and more specifically IL-8 hyperproduction.

The first published contribution of miRNA involvement in CF [8] reported that miR-126 was found to be abundantly expressed in normal lungs and that it was reduced in lungs of patients with CF. This process cannot account for the pathophysiological mechanism of changes observed in patients with CF, based on the observation of high concentrations of IL-8 depending on constitutive activation of nuclear factor kappa B (NF-κB) [15-17]. Indeed, Oglesby et al. [8] found that in patients with CF, miR-126 was strongly downregulated and expression of TOM1, which is itself a negative regulator of the NF-κB signaling pathway, was upregulated. On this basis, a decreased expression of IL-8 would be expected, in contradiction to the well-established increased IL-8 production. These observations indicate that miR-126 alone does not play a role via the TOM1 cascade and that other factors may also be involved. Other miRNAs originated from host or part of this microbioma could also be involved. For example, Rao et al. [18] reported their finding of bacterial miR-146, which binds to a receptor of the TLR family, in sputum of CF patients infected with *Pseudomonas aeruginosa*.

As for other miRNAs, it has been suggested that expression of miR-155 might contribute to the activation of IL-8--dependent inflammation in patients with CF. Clinically, overexpression of miR-155 has been observed in lung epithelial cells and in neutrophils from patients with CF [18]. Interestingly, miR-155 expression was also elevated in primary CF bronchial epithelial cells and in IB-3 and CuFi-1 CF cell lines [16, 19]. Upregulating miR-155 expression in these cells increased production of IL-8 through activation of the PI3K/Akt signaling pathway [16]. Another study confirmed these results [17] by showing that high levels of miR-155 in sterile CF cells, reduced after exposure to the anti-inflammatory cytokine IL-10 or following inhibition

of IL-1β signaling, was accompanied by a reduction of IL-8 production. These observations suggest a general role of miR-155 as an IL-8 expression regulator and consequently of the NF-κB pathway [16, 17, 19, 20]. The expression of miR-155 is also increased in patients with asthma, idiopathic pulmonary disease and acute lung injury [21]. It has been underlined that a tight control is required for the expression of a molecule such as miR-155 which is overexpressed in cancers of B-cell origin. It is noteworthy that a signaling molecule as small as miR-155 has such a dangerous potential when deregulated.

Although so far, few studies have focused on the role of miRNA as regulator of the immune response specifically in the CF context, it is of prime importance to consider other miRNAs, the expression of which is deregulated in inflammatory lungs, and which could be non-specifically modulated in CF. CF cells display a new profile of miRNAs, including high expression of miR-215 which is a strong modulator of cell cycle through the p53-signaling pathway [17]. As an example of unspecific modulation, miR-509-3p and miR-494 (which directly target CFTR expression, as described above) are overexpressed in CF bronchial epithelial cells [9]. In addition, bacterial infection and tumor necrosis factor-alpha and IL-1β exposure increase miR-509–3p and miR-494 concentrations in part via the action of the NF-kB transcriptional activator complex. These findings, together with those showing that miR-494 is upregulated in CF cells [9, 10], support the idea of a role of miRNAs in inflammatory responses in CF respiratory epithelia, either directly by activation of transcription factors such as NF-κB, or indirectly by inhibition of CFTR expression.

Another crucial aspect of the immune response in lungs of patients with CF is the dysregulation of the protease-antiprotease balance which eventually leads to bronchiectasis [22]. Much attention has been given to serine proteases, in particular elastase secreted by neutrophils massively recruited during lung inflammation in patients with CF. However, other proteases secreted by epithelial cells themselves impact airway function. Recently, involvement of cysteine proteases cathepsin (CTS) B and S, overexpressed in lungs of patients with CF, has been described [23]. CTSS is constitutively released at high levels by airways of patients with CF. Weldon et al. showed that the overproduction of CTSS in lungs of patients with CF was indirectly modulated by miR-31 in HBEs via repression of the transcription factor, interferon regulatory factor 1 (IRF1), which directly controls the *CTSS* gene [24]. It could be predicted that evidence will be brought up, showing that other proteases, such as metalloproteinases, often detected at high levels in CF patients sputum, are also regulated by miRNA-dependent mechanisms. Similarly, miRNAs could be involved in the expression of anti-proteases (e.g., α-1 antitrypsin, secretory leucocyte protease inhibitor (SLPI), tissue inhibitors of metalloproteinase 1 (TIM-1)), opening new perspectives in development of novel therapies for CF and other lung diseases.

It is quite likely that a number of miRNAs could find several target points in the network of cellular and molecular players of the inflammation, imbalanced in CF. In turn, inflammatory responses themselves drive the expression of several miRNA species that either worsen the

imbalance by increasing production of proinflammatory mediators or directly repress CFTR protein expression, forming a vicious circle.

4. Regulation of lung cell development and differentiation

Studies of miRNAs expression patterns have revealed that 27 miRNAs are differentially expressed during lung embryogenesis [25] following a characteristic pattern. miR-29a is highly expressed in late stages of lung development and in adult life [26]. In contrast, the miR-17-92 locus is highly expressed in undifferentiated lung epithelial cells and in lung cancer cells [27], and its expression progressively declines as differentiation progresses. Interestingly, miR-127 and miR-351 are transiently expressed during late phases of lung embryogenesis, first in the mesenchymal network, and then in epithelial cells. These observations suggest that miRNAs play distinct roles in the differentiation processes during the mesenchymal-to-epithelial transition.

The chronic inflammation in CF leads to irreversible airway tissue remodeling characterized by loss of multiciliated cells, gain in mesenchymal cells, goblet cells hyperplasia, and squamous metaplasia. Two miRNAs involved in multiciliogenesis have particularly attracted attention. miR-449a accumulates in bronchial epithelial cells during their transition to full differentiation [28]. Involvement of this particular miRNA in multiciliogenesis has been previously described [29]. Expression of miR-449a remains high in differentiated cells, indicating that it also plays a role in maintaining the multiciliated phenotype [29]. Conversely, expression of miR-455-3p, which negatively regulates the mucin 1 gene, is lost during the differentiation process of HBEs, reinforcing the control of epithelial (de)differentiation by miRNAs. Consequently, high expression of miR-449a and low expression of miR-455-3p can serve as biomarkers for the differentiation of bronchial epithelia. Further studies would be welcome to determine whether these miRNAs are deregulated in CF cells.

Airway epithelium dedifferentiation is part of tissue remodeling through induction of epithelial-mesenchymal transition (EMT). EMT is a process during which epithelial cells lose their phenotype, including loss of cell polarity and dissolution of cell-cell junctions, and acquire mesenchymal characteristics. Transforming growth factor (TGF)-β is a key mediator of EMT and epithelial remodeling. TGF-β signaling was shown to be increased in CF lungs [30] and several miRNAs are regulated via the TGF-β pathway [31]. Among them, miR-155 expression is reduced in human fibroblast cells upon exposure to TGF-β [32]. Although it may contribute to reduce inflammation (through its effect of IL-8 secretion in HBEs described earlier), reduction of miR-155 expression at the airway level might contribute to loss of epithelial polarity. Moreover, another study utilizing epithelial cells indicated that miR-155 plays an important role in TGF-β-induced EMT as it facilitates tight junction dissolution [33]. Because CFTR expression is important to maintain epithelial differentiation and polarity [34], in particular in CF cells [35], it could be expected that at least some of the other miRNAs controlling CFTR expression might be involved in airway remodeling as well. Likewise, other

miRNAs might non-specifically regulate epithelial differentiation in CF cells and could be involved in loss of epithelial polarity.

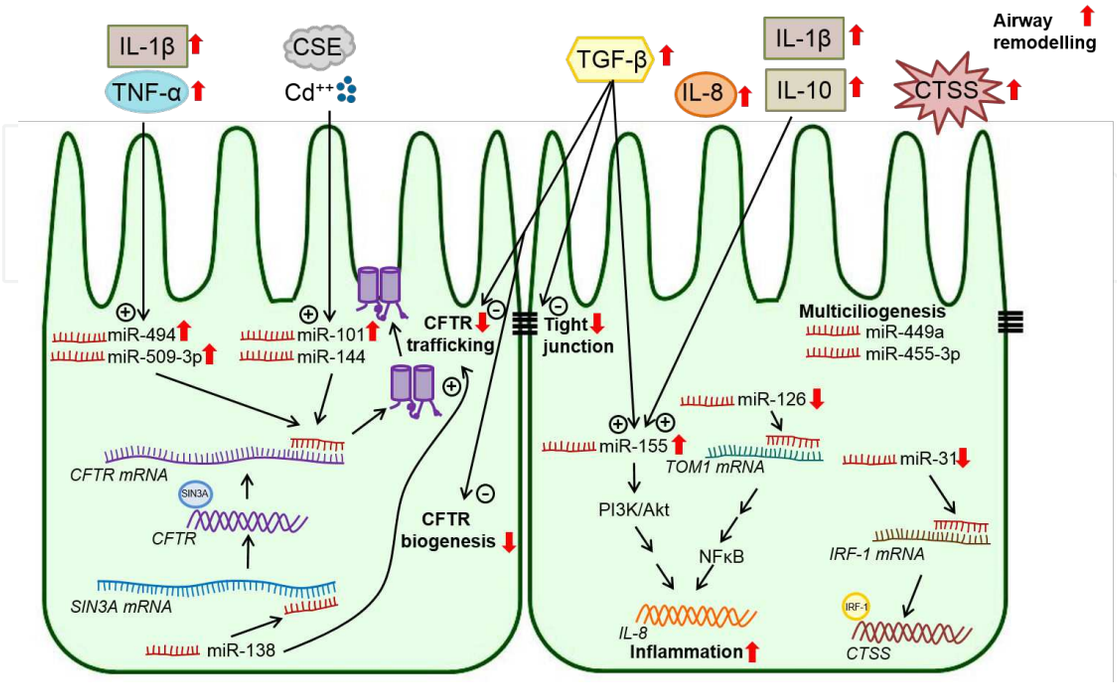

Figure 2. Main miRNAs involved in cystic fibrosis pathogenesis The left part depicts miRNA-dependent regulation of cystic fibrosis transmembrane conductance regulator (CFTR) biogenesis and trafficking. The right part zooms into the regulation of interleukin (IL)-8 production by miRNAs. miRNAs which may drive phenotypic changes on multiciliogenesis, airway remodeling and disorganization of tight junction are also represented. Red arrows depict changes reported in CF; up arrows indicate upregulated miRNAs and phenotype; and down arrows indicate miRNA and phenotype downregulation. CSE: cigarette smoke exposure; CTS: cathepsin.

MicroRNAs potentially altered and phenotypic consequences in CF are summarized in Table 1.

Target	MicroRNA	Expression	Consequences	References
CFTR	miR-101, -144, -494	Overexpressed in CF nasal and bronchial biopsies	Downregulation of CFTR expression	4,5,7,8,10
CFTR	miR-145,-223, -494, 509-3p	Overexpressed in CF bronchial brushings and CFBE41o- cell line	Downregulation of CFTR expression	3,5,9,10
SIN3A	miR-138		Corrector of CFTR trafficking Inhibition of proteosomal degradation of F508del-CFTR, Cl^- permeability	11
TOM1	miR-126	Decreased expression in CF bronchial brushing and CFBE41o- cell line	Unexplained downregulation of NF-κB	8

Target	MicroRNA	Expression	Consequences	References
SHIP1 (Pi3K/Akt)	miR-155, -125	Overexpressed in CF neutrophils, in primary CF bronchial epithelial cells and in CF cell lines (IB-3 and CuFi-1)	IL-8 hyperproduction NF-κB activation Proapoptotic p53-signalling pathway EMT via TGF-β signalling	16,17,19,20, 32,33
IRF-1	miR-31	Decreased expression in CF bronchial brushing	Cathepsin S hyperproduction	24
	miR-29a	Highly expressed in late stages of lung development and in adult life	Lung embryogenesis	26
	miR-127, -351	Transiently expressed during late phases of lung embryogenesis	Modulation of lung development	25
	miR-17-92	Highly expressed in undifferentiated lung epithelial cells	EMT	27
	miR-449a	Accumulated in bronchial epithelial cells	Multiciliogenesis, EMT	28,29
MUC1	miR-455-3p	Expressed in human bronchial epithelial cells	EMT negative regulation of mucin 1	29

EMT = epithelial mesenchymal transition

Table 1. microRNAs potentially altered and phenotypic consequences in cystic fibrosis (CF)

5. miRNAs as biomarkers and potential therapies

As in other diseases in which miRNAs have been involved, such as asthma, COPD, idiopathic pulmonary fibrosis, cancer and diabetes, further analyses of miRNA expression-function relationships will very likely reveal new genetic factors that could be targeted in therapy. There is also a great hope that miRNAs could be used as diagnostic markers and represent new prognostic factors that might influence the course of the CF disease.

The pathophysiology of CF is very variable from patient to patient and is only partly explained by the *CFTR* genotype. Two recent studies raised the hypothesis that profiling serum miR-Nome could identify miRNAs as potential prognostic biomarkers [16, 36]. First, elevated miR-155 serum levels have been detected in patients with CF, possibly reflecting its high expression in CF airway cells [16]. Second, a prototype study by Cook et al. has suggested that serum miRNAs could be used as diagnostic markers in CF liver disease [37]. Profiles of circulating miRNA levels in patients with CF liver disease were compared to those of CF patients without liver disease and of non-CF controls. For the first time, changes in circulating miRNA levels were identified in CF and they were correlated with disease status, suggesting that serum miRNA analysis may help predict early onset of hepatic fibrosis in CF [36]. It could

be expected that new biomarkers of the course of the CF lung disease will be identified in the coming years.

It can also be foreseen that single nucleotide polymorphisms (SNPs) in the 3'UTR of miRNA-targeted genes, in particular CFTR, may explain phenotype variability. As an illustration, an SNP (c.*1043A>C) was identified in the 3'UTR of CFTR in a patient with a CFTR-related disease [38]. CFTR-related diseases are clinical entities associated with CFTR dysfunction but that do not fulfil diagnostic criteria for classical CF (e.g., Congenital Bilateral Absence of Vas Deferens (CBAVD), chronic pancreatitis and disseminated bronchiectasis). This SNP was located within the binding site of two miRNAs including miR-509-3p (shown otherwise to directly target CFTR mRNA), and experimental data suggested that it might impair the regulation of gene expression. That could explain the mild phenotype, as this SNP would act as a mild mutation. Consequently, polymorphisms in the CFTR 3'UTR may play a role in the observed heterogeneous phenotype. Molecular analysis of the 3'UTR of CFTR could therefore be performed as a differential diagnostic tool in patients presenting with suggestive clinical symptoms of CF but no mutation in the *CFTR* gene per se.

Modulating miRNA expression in vivo looks very appealing for developing new CF therapies [38]. Indeed miRNAs are short and need to be delivered only to the cytoplasm, as opposed to nucleus delivery required for DNA-based constructs. Moreover, miRNA-based therapies have several advantages over gene therapy strategies aiming at restoring CFTR expression. First, miRNA modulators are likely to target multiple genes in the context of a deregulated network. However, this might also be a major drawback as potential off-target effects may cause adverse phenotypes. Second, as in all cases of gene therapies, tissue-specific delivery remains a major issue in miRNA-based therapies. An interesting approach has been used by McKiernan et al. to successfully deliver miRNA replacement therapy in CFBE41o- cells [39]. They demonstrated that polyethyleneimine nanoparticles complexed with pre-miR-126 resulted in significant knock-down of TOM1, previously described as a direct target of miR-126 [8]. This result shows that polymeric nanoparticles may be used to effectively deliver miRNA replacement therapy with no adverse effects and may present a strong advantage in comparison to virus-based delivery strategies. As for any other disease for which miRNA-based therapy is considered as an attractive new option, factors controlling the stability of the miRNAs, the delivery systems and the off-target effects of miRNA-based therapies represent strong challenges for the future of development of such drugs.

6. Limitations in translating miRNAs from deeper knowledge of their role in pathogenesis to development of new therapies

Altogether, it is crucial to highlight important experimental considerations regarding miRNA investigations in CF. Expression profiles of miRNAs in human CF bronchial tissue often differ from study to study. The differences can be explained in part by the selection of tissue material. Indeed, the presence of inflammatory cytokines or non-resident, migratory cells (neutrophils, macrophages, etc.) or both infiltrating airway epithelium generate a non-negligible degree of heterogeneity in biopsy samples. Similar issues can be faced when analyzing miRNA expres-

sion in human primary HBEs cultures. miRNA expression is strongly dependent on cell origin and differentiation state and on culture conditions. Accordingly, the air-liquid interface condition, which favors differentiation towards the epithelial phenotype, might influence miRNA expression. Moreover, although cultured, undifferentiated HBEs are quite homogenous, when switching to air-liquid interface condition, not only ciliated cells but also basal and goblet cells are found. In summary, any heterogeneity in cell population is likely to bring bias in miRNA expression profiles.

The large discrepancy among miRNA studies on CF disease may also indicate tissue- or organ-specific expression patterns of miRNAs [40]. Consequently, miRNA gene targeting may be variable in different tissues and organs as well. This point is particularly relevant in CF, a multisystemic disease. Similarly, studies aimed at profiling miRNAs in established CF mouse models may show differences that could be species specific. Substantial differences in technical approaches and statistical analyses are also important factors of variability and lack of reproducibility among studies.

Moving towards translation from bench to bedside, practical limitations including degradation and inactivation by nucleases, efficacy of intracellular delivery, short plasma elimination rates, renal and dose-limiting hemodynamic toxicities may hamper development of miRNA-based therapies. As miRNAs have pleiotropic intracellular effects on multiple signal transduction pathways, either single or combined therapies targeting CFTR expression, modulation of inflammation and cell differentiation might eventually be considered. This development could prove lengthy and full of traps and pitfalls before reaching a stage of translation to the clinical setting.

7. Conclusion

The study of miRNA in CF is still at an embryonic stage. To date, several studies have determined profiles of miRNAs in samples from target tissues such as lung and nasal biopsies, primary bronchial epithelial cell cultures or epithelial tissues from relevant mouse models. They have generated some indirect data suggesting a significant role of miRNA in controlling lung development, CFTR expression, as well as inflammation signaling pathways in CF.

The preliminary findings reviewed here form a solid basis for growing interest in miRNAs in CF. The possibility that they may act as phenotype modifiers and that they could be used as diagnostic and prognostic biomarkers looks very attractive. With no doubt, further research on miRNA biogenesis and function in CF will expand widely in the coming years, shedding new light on disease variability and paving the way to innovative therapies.

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