miRNA in Regulating CFTR

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The Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is a cAMP-activated ion channel that mediates transepithelial Cl^- and HCO^- secretion in fluid transporting epithelia. In the airway, a thin layer of fluid, known as the airway surface liquid (ASL), acts as a medium to facilitate ciliary function and allow for the overlying mucus layer to glide from the distal lung to the trachea where it can be expelled from the lung. *CFTR* gene mutations impair regulation of the transport of Cl^-, HCO^-, Na^+, and water, and alter the volume and composition of the luminal contents of the airway, intestine, and biliary tree, leading to muco-obstructive disease cystic fibrosis (CF). Deletion of phenylalanine in position 508 (p.F508del) of the CFTR gene is present in at last 70% of CF patients. We discuss the role of micro(mi)RNAs in regulating CFTR expression and function in health and disease.

Keywords: micro RNA ; CFTR ; Cystic Fibrosis

1. Introduction

The expression of the *CFTR* gene is tightly regulated in a temporal and tissue-specific manner [1][2]. Gillen et al. first reported the role of miRNAs in CFTR expression [3]. The validated CFTR inhibitors miR-101, miR-145, and miR-384 play an essential role in the switch from a strong fetal to low postnatal CFTR expression [4]. Interestingly, miR-101 negatively regulated CFTR in the adult airway cell lines but did not affect CFTR in the fetal bronchial epithelial cells. These data demonstrate that miRNAs control the temporal expression of CFTR. In the postnatal airway, the CFTR protein is abundant in the submucosal serous gland cells, much less abundant in multi-ciliated surface epithelial cells, and highly expressed in the newly identified ionocytes [5][6][7]. The role of miRNAs in controlling the cell-type-specific expression of CFTR in the airway epithelium is practically unknown.

2. Role of miRNA in Regulating CFTR

Many miRNAs have been experimentally validated as CFTR inhibitors [3][4][8][9][10]. miR-101 and miR-494 markedly repressed CFTR expression alone and had a more substantial synergistic effect [11]. Other groups reported synergistic inhibitory effects on CFTR for the miR-145, miR-223, miR-384, miR-1246, and miR-494 or miR-509-3p together with miR-494 [3][8][9]. A reciprocal regulation was proposed that a decreased CFTR Cl^- channel activity may contribute to the overexpression of miR-145, miR-223, and miR-494 in the CF airway [3]. These data suggest that the severity of CF airway disease can be influenced by conditions that affect the active pools of the synergistically acting miRNAs. Enhancing the affinity of CFTR mRNA for miRNA binding is an exciting novel mechanism of CF that may explain why *CFTR* gene mutations are not identified in up to 10% CF alleles. Amato et al. reported a single nucleotide polymorphism (SNP) in the CFTR 3'UTR that increases the binding affinity of validated CFTR inhibitor miR-509-3p and reduces expression of CFTR protein, acting as a mild CFTR mutation [12]. Endale Ahanda et al. identified gene polymorphisms in the miR-99b/let-7e/miR-125a cluster that modulate the expression of these miRNAs [13]. Two of the polymorphisms in a cohort of p.F508del CF patients could modulate miRNA maturation and therefore impact the miR-99b/hsa-let-7e/hsa-miR-125a activity, acting as non-CFTR gene modifiers in CF. They may help to explain the variable severity of lung disease among CF patients with the same genotype.

The *TGF-β1* gene is a known non-CFTR modifier in p.F508del CF patients. Two SNPs present in ~40% of *F508del* homozygous patients, increase TGF-β1 protein levels, correlate with more severe lung disease, and exacerbate the damaging effects of secondhand smoke in CF patients [14][15]. Besides, *Pseudomonas aeruginosa* infection and reduced nutrition increase TGF-β1 levels in p.F508del homozygous patients [16][17][18][19]. Independent of the underlying cause, high TGF-β1 levels are strongly associated with poor outcomes [20][21][22][23][24][25]. Thus, TGF-β1 may represent a prevalent ASL inhibitor and an antagonist limiting the residual and corrected CFTR activity in CF patients. TGF-β1 inhibits CFTR mRNA level and reduces the full beneficial effects of CFTR correctors in human airway epithelial cells [26][27][28]. Although TGF-β1 is a transcriptional regulator, current data show that its inhibitory effect on CFTR is mediated post-

transcriptionally via miRNAs, including miR-145 and miR-143 [29][30][10][31]. TGF-β1 changes the expression of many miRNAs, including those validated as CFTR inhibitors [29][31][32]. However, the total cellular miRNA level does not correlate with the inhibitory effect on a target gene. In agreement with this view, we have recently shown that TGF-β1 recruits specific miRNA to RISC, independently of how it affects their total cellular levels [29]. Only the miRNAs validated as CFTR inhibitors and recruited by TGF-β1 to RISC, including miR-143 and miR-145, would mediate the TGF-β1 inhibition of CFTR mRNA. This study provides another novel observation that the cellular environment of chronic lung disease, including CF, contains additional factor(s) required for the TGF-β1-mediated decay of CFTR mRNA [29]. Data showing that TGF-β1 did not inhibit CFTR mRNA in primary human airway epithelial cells from lungs without chronic disease despite recruiting miR-145 to RISC and increasing the total cellular miR-145 levels support the conclusion. These data emphasize the complexity of the TGF-β1-miRNA axis and its context-specific effects. TGF-β1 plays a significant role in the pathogenesis of other forms of lung disease, including chronic obstructive pulmonary disease (COPD), the third leading cause of death in the US, where it causes acquired CFTR dysfunction by cigarettes smoke exposure [25][33][34][35][36][37]. Environmental pollutants, including cigarette smoke, also increase TGF-β1 levels and raise the risk of sinopulmonary disease in carriers of the *CFTR* gene mutations (15,000,000 people in the US), compared to the general population [38]. The SNPs associated with high TGF-β1 levels may also contribute to the acquired CFTR dysfunction. We have shown that TGF-β1 inhibits CFTR mRNA in human bronchial epithelial cells from COPD and idiopathic pulmonary fibrosis (IPF) lungs [29]. These data suggest that miRNAs may also carry out the TGF-β1 repression in these conditions. Dutta et al. provided evidence for the role of TGF-β1 and miR-145 in cigarette smoke-induced acquired CFTR dysfunction [31]. Cigarette smoke exposure is associated with a specific signature comprised of a network of miRNAs and proinflammatory signaling cascades, leading to decreased pulmonary function [39]. Avoiding cigarette smoke exposure is the only valid measure known to date to prevent the harmful effects mediated by these miRNAs.

Some miRNAs induce CFTR expression by targeting transcriptional repressors. For example, the miR-138 mimic restored the p.F508del-CFTR expression and function by downregulating the expression of the highly conserved transcriptional repressor SIN3A [40]. Although miR-138 may have a positive effect on CFTR protein abundance and the CFTR Cl^- channel function, overexpression of other genes would be expected as a result of the miR-138-mediated inhibition of SIN3A. Thus, miR-138-based therapy for CF is not feasible. By contrast, blockade of the MRE in CFTR 3'UTR by TSBs can precisely restore the CFTR Cl^- channel activity in CF bronchial epithelial cells. De Santi et al. recently showed that TSBs directed against the miR-223-3p and miR-145-5p MREs in the CFTR 3'UTR, encapsulated in poly-lactic-co-glycolic acid (PLGA) nanoparticles and delivered to the airway in an aerosolized form, increased CFTR expression and function in CF bronchial epithelial cells [32]. Thus, TSBs emerge as potential therapeutics precisely and specifically eliminating the inhibitory effects of miRNA on CFTR, allowing the full potential of the FDA-approved CFTR modulators in the CF airway. Moreover, the prevention of the hypoxic milieu of the muco-obstructive airway disease in CF may enhance the efficacy of CFTR correctors by preventing miRNA-200b from directly targeting the CFTR mRNA [41].

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