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Developmental control of CFTR: from bioinformatics to novel therapeutic approaches.

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Developmental control of *CFTR*: from bioinformatics to novel therapeutic approaches

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Celebrating the 25th anniversary of identifying the genetic cause of cystic fibrosis, tremendous progress has been made in the understanding and the treatment of this complex, but still fatal disease [1]. Despite advances, several key questions on the origins and pathomechanisms of CF remain poorly understood. For instance, the precise genetic and epigenetic mechanisms that regulate expression and stability of the *cystic fibrosis transmembrane conductance regulator (CFTR)* gene remain incompletely defined.

CFTR gene expression is a carefully controlled process that is spatially and temporally regulated. Notwithstanding the extensive research that has been carried out on *CFTR* over the past few decades, developmental control of *CFTR* expression is still poorly understood [2]. Transcription of *CFTR* starts at distinct sites depending on the tissue or developmental stage and is positively regulated by a selection of transcription factors, including C/EBP proteins and FOXA factors. Beyond that, *CFTR* is controlled post-transcriptionally by microRNAs (miRNAs). miRNAs are regulatory factors involved in most biological processes and it is

becoming increasingly clear that they play a key role in the development and manifestations of CF lung disease [3-13]. These small non-coding RNAs act post-transcriptionally to inhibit protein production. Their involvement in the pathogenesis of CF lung disease stems from the fact that their expression is altered *in vivo* in the CF lung due to intrinsic and extrinsic factors; to date defective chloride ion conductance, endoplasmic reticulum stress, inflammation and infection have been implicated in altering endogenous miRNA expression in this setting.

With their recent study, Viart *et al.* now expand substantially our understanding of how *CFTR* expression is regulated [14] (Figure 1). The authors identified regulatory elements that participate in *CFTR* downregulation, particularly FOXA1, FOXA2 and C/EBPα. Using bioinformatics, they further identified four new AU-rich elements (ARE) and experimentally tested their roles in regulation of *CFTR* expression. Following a series of intricate studies, ARE-5698 was found to play a role in destabilizing *CFTR* mRNA. Regarding miRNAs predicted to bind to the *CFTR* 3'UTR, miR-101 had the strongest repressive effect. By comparing miRNA expression profiles of adult and foetal lungs, three specific miRNAs (miR-145, miR-150 and miR-451) were found to have a temporal effect, being significantly up regulated in the adult lung and therefore contributing to downregulation of *CFTR*.

With a view to therapeutics that might enhance *CFTR* expression, miRNA binding-blocker oligonucleotides (MBBOs, also known as target site blockers/protectors or miRNA masks) were designed. The function of an MBBO is to prevent binding of a miRNA to one specific mRNA target. Here MBBOs for several miRNAs that bind the *CFTR* 3'UTR were tested *in vitro* and *ex vivo* using non-CF and p.Phe508del homozygous CF primary human nasal epithelial cells grown at an air-liquid interface. MBBOs targeting the miR-145 and miR-101 sites were most effective, as they stabilised *CFTR* mRNA and enhanced CFTR protein expression. Finally, both MBBOs significantly enhanced CFTR function in CFBE41o- cells.

This report adds significantly to our existing knowledge regarding miRNA regulation of *CFTR* [5-10, 13] and the developing field of miR-based CFTR therapeutics [15]. The data confirms previous studies implicating miR-145 and miR-101 as important modulators of *CFTR* expression [5, 6, 8, 9] and build on these by demonstrating how MBBOs based on these miRNAs can affect *CFTR* gene expression and CFTR protein function. The authors suggest that the MBBOs may be developed as tools for CFTR correction in people with CF. Indeed this is an appealing option given that MBBOs are less likely to have off-target effects compared to some other miRNA inhibition strategies [reviewed in 16].

Although there are a series of novel findings from this paper, the work also raises a number of important questions for further investigation. The CF lung is inherently associated with microbial colonisation. Whether infection impacts upon the newly described transcriptional and post-transcriptional mechanisms controlling *CFTR* expression remains elusive. This will be particularly important for the further development of the MBBOs as therapeutics for CF. Previous studies have reported how infection and inflammation affect the expression of miRNAs, including those that regulate *CFTR* [9, 13]. This suggests that higher than normal levels of MBBOs may be required to ensure an inhibitory effect on endogenous miRNA activity. Nonetheless it will be a very exciting development when miRNA-modulating drugs for CF advance to the stage of clinical studies. A second point worth considering is the relevance of Viart *et al.'s* findings beyond CF. Separate sets of studies have clearly shown that cigarette smoke affects *CFTR* and miRNA expression. Although the mechanisms responsible for altered CFTR expression in, for example, the COPD lung are not restricted to miRNA-mediated effects [17], miR-101 in particular is known to be increased by cigarette smoke extracts (CSE) and directly impacts on *CFTR* expression [8]. Finally, it is becoming increasingly evident that dysfunctional CFTR as a result of cigarette smoking can contribute to the pathophysiology of a range of extrapulmonary disease conditions, such as

chronic pancreatitis, male infertility and cachexia [18]. Expanding upon the findings from airway epithelial cells reported here into non-lung cells that express CFTR may yield novel therapies for these disease conditions beyond CF.

FIGURE LEGENDS

Figure 1. *Transcriptional and post-transcriptional regulation of CFTR*. In the adult lung (A) the transcription factors FOXA1/2 and C/EBPα negatively regulate *CFTR* gene expression whilst (B) ARE-5698 destabilises *CFTR* mRNA and miR-145 and miR-101 block *CFTR* expression. MBBOs targeting the miR-145 and miR-101 sites can restore *CFTR* expression.

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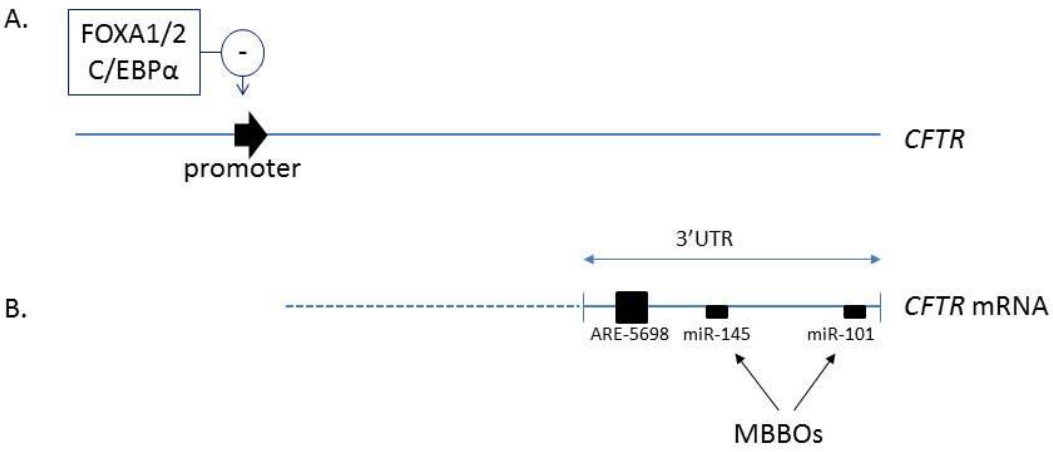
A.
FOXA1/2
C/EBPα
-
promoter
CFTR
B.
3'UTR
CFTR mRNA
ARE-5698
miR-145
miR-101
MBBOs