

EDITORIAL



Implications of non-coding RNA on Cystic Fibrosis: new therapeutic avenues

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“Some non-coding RNAs are highly likely to play a role in disease pathogenesis and elucidation of their functions would enhance our understanding of basic CF biology.”

Although cystic fibrosis (CF) occurs due to a recessive mutation in a single gene, gene therapy to correct this defect has remained unrewarding. In fact, until recently the scientific and medical community has only been able to treat the varying symptoms of the disease; but recent advances offer much hope for the treatment of the underlying genetic defect. CF predominates in Caucasians compared to other races and Ireland has the highest carrier rate in the world (1 in 19) and a

CF prevalence of 2.98 per 10,000 live births. This is four times higher than that of the USA or the European Union (EU) average, where the cost of CF healthcare approximates to €0.6 billion per annum [1,2]. While advances in recent years have improved treatment and lengthened the median survival of people with CF, there is still no effective cure.

CF is a lethal autosomal recessive disorder affecting many organs. However, its pulmonary manifestations are responsible for

the associated high morbidity and mortality. The disease is characterized by chronic lung infection and inflammation, leading to irreversible lung damage and death. Over 1,900 cystic fibrosis transmembrane conductance regulator gene (*CFTR*) mutations have been identified to date, yet a deletion of phenylalanine at position 508 of the *CFTR* protein is the most frequent mutation, and accounts for approximately 70% of the alleles in CF worldwide. The *CFTR* gene encodes an

ATP-regulated chloride channel and is present within the apical surface of epithelial cells throughout the body. The development of chronic inflammatory lung disease is typically the primary manifestation in people with CF, with additional disease in other organ systems including pancreatic insufficiency, sweat electrolyte imbalance and male infertility [3]. Some of the major determinants of CF lung disease include a decreased airway surface liquid volume, increased mucus viscosity, chronic microbial colonisation, an impaired protease-anti-protease balance and increased pulmonary inflammation.

Although great strides have been made in recent years in developing a cure for CF through the discovery of potentiator and corrector drugs, there is still some way to go to achieve a cure for CF. Importantly, not all CF sufferers can benefit from the current drugs that are being developed. Therefore there is a need to explore other therapeutic strategies for CF that could be used as stand-alone drugs or in conjunction with inflammation/infection-directed and/or CFTR corrector therapies and a move toward personalized therapy could greatly enhance our treatment of CF. Although CF is a disease caused by a mutation in a single gene – *CFTR* – it is well-known that other disease-modifier genes exist which alter the severity and course of the disease. Both chronic bacterial infection and the underlying genetic defect result in a pro-inflammatory environment within the lung but other factors can also influence CF lung disease severity. The importance of non-coding RNA in the pathogenesis of CF lung disease is only beginning to be realized.

It is becoming increasingly apparent that much of the human genome encodes non-coding RNA (ncRNA), and that only a little more than 1% of human RNA actually encodes proteins. ncRNA is a super-class of non protein-coding RNA, which comprises a large proportion of cellular RNA. In recent years exciting new roles have emerged for these molecules. The two most widely studied ncRNA sub-classes are microRNAs (miRNAs) and long non-coding RNAs (lncRNAs); both have vital roles in the transcriptional and post-transcriptional regulation of almost the entire genome. There are ~2,000 human miRNAs and their principal function is the post-transcriptional repression of gene translation [4]. Up to 30,000 lncRNAs may exist in humans and several of these transcripts have been functionally implicated in the regulation of gene expression at almost every level due to their ability to act as bridging molecules between DNA, RNA and/or proteins.

Expression levels of ncRNAs vary greatly between cells and tissues, and aberrant levels have been associated with many diseases in humans. We performed the first miRNA and lncRNA profiling studies in CF bronchial brushings and identified that ncRNA expression patterns are significantly altered in people with CF [5,6]. Numerous miRNAs demonstrate altered expression between CF and non-CF bronchial epithelium and the altered miRNAs are predicted to regulate various processes including innate immunity, inflammation, endoplasmic reticulum (ER) stress and ion conductance [reviewed in 7]. Regarding lncRNAs, our study identified over 1,000 lncRNAs with significant differential expression between CF

and non-CF individuals; however the roles of the altered lncRNAs within the context of CF remain largely unexplored. Some of these lncRNAs are highly likely to play a role in CF disease pathogenesis and elucidation of their functions would enhance our understanding of basic CF biology.

Within the nascent field of 'ncRNA and CF', areas that deserve further study include translational studies that will provide a greater insight into the expression patterns of ncRNAs in larger groups of people with CF, of different ages, with different *CFTR* genotypes and co-morbidities, and those undergoing specific treatments. This information, coupled with functional studies would help to the identify ncRNAs that contribute to CF pathogenesis, and thus represent novel therapeutic targets.

Delivery of therapeutics that target ncRNA using nano- and micro-technology directly to the respiratory tract is a promising modality for non-invasive local treatment of CF respiratory disease. Whilst we are beginning to learn more about miRNAs in CF and to explore whether they represent novel therapeutic targets, similar advances regarding lncRNAs and CF lag behind. In the case of the aberrant overexpression of a miRNA, various strategies are currently being investigated including the use of modified antisense oligonucleotides, specifically antagomiRs [8]. However one caveat regarding their use, specifically those with a phosphorothioate backbone, is that they can activate platelets [9]. Currently the most advanced miRNA therapy is a Locked Nucleic Acid-based inhibitor of miR-122 (Miravirsen, SPC-3649, Santaris Pharma, Denmark)

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for hepatitis C infection – unusually this therapy does not work by blocking miR-122 binding to the 3' untranslated region (UTR) of a target mRNA, rather it prevents miR-122 binding to the 5'UTR of the hepatitis C viral RNA and promoting its replication. Where underexpression of particular miRNAs is involved, these may be introduced into affected cells as pre-miR mimics. That miRNAs can regulate multiple different mRNA targets, usually belonging to related signalling networks, is part of their power as regulatory factors. The downside of this redundancy is that by targeting a single miRNA, invariably more than one target mRNA will be affected. However for one particular application, namely inhibition of miRNA function, it is possible to custom-design specific miRNA-target mRNA inhibitors, commonly referred to as target-site blockers.

Similar to other nucleic acid-based therapeutics, effective anatomical and cellular delivery of miRNA modulators is a major hurdle to their clinical and commercial development. At a cellular level, small RNAs that utilize the RNA interference machinery need only be delivered to the cytoplasm where the RNA-induced silencing complex is active, rather than to the nucleus as is required by other methods, making the approach simpler. These negatively charged molecules

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can be targeted by endogenous nucleases, cleared by glomerular filtration, display poor cell membrane permeability and limited endolysosomal escape. Thus a range of protective vectors have been explored for their delivery to overcome these barriers, including viral and non-viral vectors [reviewed in 10]. Lentiviral or other viral approaches require cell specific promoters and are not wholly applicable for human disease unless transducing cells *in vitro*; e.g. therapies for chronic granulomatous disease and Wiskott-Aldrich syndrome. Our research focuses on the use of polymeric particles which, when engineered appropriately, can facilitate intracellular delivery, enhance *in vivo* stability and display targeted delivery to specific cell types within the lung.

We have successfully used synthetic polyethylenimine (PEI) cationic polymers to deliver miRNAs into bronchial epithelial cells [11]. As toxicity and lack of biodegradability can be an issue with PEI, we routinely use high content analysis and multiparameter toxicity testing to assess these potential problems. Nevertheless the spotlight is now turning towards the use of PEI derivatives and other biocompatible, biodegradable polymers such as chitosan, poly(lactide) (PLA) or poly(lactide-co-glycolide) (PLGA). PLGA can be used to

generate a wide range of particles from nanoparticle (~10–1000 nm) to microparticle (1–250 µm) sizes. Consequently, PLGA particles encapsulating nucleic acids can be developed for both inhalation and targeting to the lower respiratory tract. Modified PEGylated-PLGA-based nanoparticles have also been designed to decrease potential inherent proinflammatory or toxic properties [12].

Local delivery to the CF lungs is one of the most promising approaches for bringing miRNA-technologies to the clinic. Inhalation offers tissue-specific targeting of the miRNA modulator and minimal systemic exposure thereby diminishing the risk of off-target effects. Recent evidence indicates that polymeric particles can offer a biocompatible and efficient means of delivering miRNA modulators effectively to cells in culture, in order to modulate gene expression, and thereby facilitate clinical translation [13]. Nonetheless, in our experience caution is required in the extrapolation of uptake studies, and downstream functional assays are ultimately required to determine the efficacy of each system. But the CF lungs represent significant anatomical and pathological barriers to inhaled medicines, due to obstructed airways being covered with thickened mucus and blocked by mucus plugs. In order to develop effective therapeutics for local aerosolised delivery to the CF lung, their efficacy in mucus-producing air-liquid interface cultures, primary CF airway epithelial cell and/or alveolar macrophage cultures and ultimately in animal models of CF are required. The complex branched anatomy of the airways also means that inhaled medicines require an

effective device to deliver them to their site of action. The recent development of advanced nebulisers, e.g. vibrating mesh devices, enables much more efficient delivery of nanomedicines to the lungs.

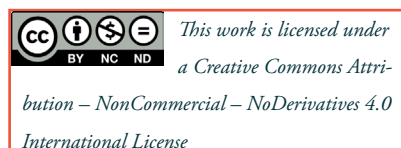
Drug–device combinations are increasingly favored by regulators worldwide in the drive for more reliable and reproducible aerosol-mediated drug delivery. Early pairing of an experimental formulation with a proven and commercially viable aerosol generator technology serves to de-risk a project by providing continuity of technology throughout the project. It also provides early insight into formulation design. Fundamentals such as drug activity post-nebulization and potential delivered dose to the patient's lung, can be characterized early and rapidly, and so can drive formulation design with respect to dose concentration, dose volume, patient interface and device choice. Further, device selection appropriate to the intended patient population needs consideration. In the CF population, where nebulizer therapy can last up to 8 hours per day for some patients, it is paramount that the chosen device does not increase that

burden. Appropriate device selection paired with appropriate patient interface and optimal dose characteristics can increase patient compliance, a critical determinant of therapeutic utility. With refinement, these platforms may become important tools in the delivery of miRNA-based therapeutics for the treatment of CF lung disease.

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