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Original Article

Translational research to enable personalized treatment of cystic fibrosis

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Abstract

Translational research efforts in cystic fibrosis (CF) aim to develop therapies for all subjects with CF. To reach this goal new therapies need to be developed that target multiple aspects of the disease. To enable individuals to benefit maximally from these treatments will require improved methods to tailor these therapies specifically to individuals who suffer from CF. This report highlights current examples of translational CF research efforts to reach this goal. The use of intestinal organoids and genetics to better understand individual assessment of CFTR modulator treatment effects to ultimately enable a better personalized treatment for CF subjects will be discussed. In addition, development of viral vectors and non-viral synthetic nanoparticles for delivery of mRNA, sgRNA and DNA will be highlighted. New approaches to restore function of CFTR with early premature termination codons using nanoparticle delivery of suppressor tRNAs and new insights into mechanisms of airway epithelial repair will be reviewed as well. The state-of-the-art approaches that are discussed in this review demonstrate significant progress towards the development of optimal individual therapies for CF patients, but also reveal that remaining challenges still lie ahead.

Keywords: Translational research; Cystic fibrosis; CFTR modulator; Personalized medicine; Gene therapy; Nanoparticles; tRNA

1. Introduction

People with cystic fibrosis (CF) have two mutations in the *cystic fibrosis transmembrane conductance regulator* (*CFTR*) in which over 2000 variants have been described to date (www.genet.sickkids.on.ca/). Severe loss-of-function mutations in *CFTR* lead to multi-organ disease, most clearly characterized by abnormal secretions and mucus plugging in the lungs and the gastro-intestinal tract of these patients [1].

Therapeutic strategies that target the various disease manifestations of CF has enabled a better quality of life and life expectancy for people with CF over the past decades [2]. Excitingly, the last ten years has led to the development of new pharmacotherapies that can (partially) repair CFTR function in a mutation-specific manner, i.e. CFTR modulators, thereby targeting directly the primary defect of the disease [3–6]. Current CFTR modulators can only robustly restore CFTR function in a minority of CF subjects, although next generation CFTR modulators are showing promise of improved efficacy in vitro and in vivo (Press release obtained from: http://investors.

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vrtx.com/releasedetail.cfm?releaseid=1033559). Still, restoration of the severe conformational defect of the most common mutation p.Phe508del (F508del-CFTR) presents significant challenges and modulators of the more rare mutations remain to be addressed. Moreover, even when a treatment is available and shows efficacy on average, considerable heterogeneity in response independent of the CF-causing mutation is observed [5,6], suggesting all patients do not benefit equally.

To optimize outcomes for all individuals with CF, we need an extensive portfolio of therapeutic approaches that will work in mutation-dependent and independent manners, paired with modalities to match therapies to individual patients. Here, we discuss current state-of-the-art examples of translational research in CF, covering new methodologies to enable improved patient stratification and prediction of CFTR modulator responses, as well as the preclinical development of new mutation-dependent and independent therapeutic approaches.

2. Patient stratification approaches for CFTR modulators

2.1. Stem cell cultures as living biomarkers of treatment response

In vitro-based assays using cultured stem cells from individuals with CF can assist in clinical decision making regarding the choice of treatment. One such approach encompasses the use of the forskolin-induced swelling (FIS) assay of intestinal organoids [7,9] that could serve as an in vitro biomarker for the prediction of in vivo response-to-therapy [8]. Recent work has demonstrated that FIS is fully CFTR-dependent and associated quantitatively with pulmonary function improvement (FEV_1) measurements of published clinical trial data [9,10]. Moreover, organoids of subjects with rare *CFTR* mutations have also been used to successfully select in vivo responders to ivacaftor (VX-770, Kalydeco®) based on their in vitro organoid responses to this drug [10].

Unpublished work focusing on 36 paired in vitro and in vivo measurements of various CFTR modulators demonstrated that in vitro FIS responses to CFTR modulators significantly correlated with in vivo treatment indicators, such as changes in pulmonary function (percent predicted FEV_1) and sweat chloride concentration (SCC). This is the first large dataset showing that an in vitro assay in biobanked adult stem cells from individuals can be used to predict in vivo drug efficacy. This data supports the further exploration of FIS as a predictive tool to identify drug responsive CF subjects for current and future CFTR modulators (and combinations thereof) in an individual-specific manner.

Intestinal organoids are stem cell cultures that can be hugely expanded while retaining patient-specific CFTR modulator response [9,10]. This large in vitro expansion capacity facilitates individual drug screening efforts, which could be important for people with rare *CFTR* mutations. Initially this assay had been set-up in 96-well plates [9], but recently it was developed into a 384-well based high-throughput screening format. Currently, a project in the Netherlands is ongoing to facilitate individual drug screening for up to 1500 FDA approved drug compounds, and all academically available CFTR modulators, for all people with rare to ultra-rare *CFTR* mutations. Approximately 90% of eligible patients responded (>150 individuals), and the first patient-specific screens revealed clear hits as well.

In conclusion, new stem cell culture and assay technology has enabled the development of in vitro living biomarkers for CFTR modulators. Unpublished data demonstrates clear relations with in vivo CFTR modulator response indicators. This translational assay also enables the screening of drug efficacy at the individual level at a scale and efficiency that has not been possible before. As such, the ex vivo testing of CFTR function in patient-derived tissue such as intestinal organoids offers important advantages for the development of personalized medicine applications in the context of CFTR modulators.

2.2. Genetic modifiers of CFTR modulator efficacy

Solute carrier family 26 member 9 (SLC26A9) is an anion channel present in epithelial cells [11,12] that interacts with CFTR in airway cells, thereby enhancing its functional expression [13–15]. In individuals with severe *CFTR* mutations, single nucleotide polymorphisms (SNPs) upstream and intronic to *SLC26A9* have been associated with intestinal obstruction at birth (meconium ileus [16]) and CF-related diabetes [17] in genome-wide association studies (GWAS) by the International CF Gene Modifier Consortium (ICFGMC). Subsequent studies demonstrated that SNP rs7512462 that marks the associated *SLC26A9* locus explains significant variability in newborn screened trypsinogen [18], and that SLC26A9 may contribute to CF-related diabetes [19] and meconium ileus (unpublished) through variation in gene expression in the exocrine pancreas.

SLC26A9 was not associated with lung function in the most recent GWAS by the ICFGMC [20]. Notable differences in SLC26A9 mRNA levels between late gestation and adult stages in opposing directions were shown in wild type murine pancreas and lung tissue [21] suggesting that the SLC26A9 – CFTR relationship may differ over time and across tissue. To support this hypothesis, Strug et al. showed that individuals with a p.Gly551Asp (G551D-CFTR) allele displayed association between lung function and rs7512462, both before and after treatment with ivacaftor. Untreated individuals homozygous for F508del-CFTR, did not show association with rs7512462. Forskolin-stimulated currents from primary human bronchial epithelial monolayers derived from 11 individuals homozygous for F508del showed improved CFTR function upon exposure to the corrector lumacaftor (VX-809) with additional rs7512462 C alleles. These observations suggest SLC26A9 requires cell-surface CFTR to modify disease outcomes in the lungs.

In summary, there is compelling evidence for *SLC26A9* as a modifier in several CF-affected organs. Current data suggests both time and tissue-specific effects that require delineation but highlight the potential for SLC26A9 as a complementary

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