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CFTR processing, trafficking and interactions☆

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ABSTRACT

Mutations associated with cystic fibrosis (CF) have complex effects on the cystic fibrosis transmembrane conductance regulator (CFTR) protein. The most common CF mutation, F508del, disrupts the processing to and stability at the plasma membrane and function as a Cl^- channel. CFTR is surrounded by a dynamic network of interacting components, referred to as the CFTR Functional Landscape, that impact its synthesis, folding, stability, trafficking and function. CFTR interacting proteins can be manipulated by functional genomic approaches to rescue the trafficking and functional defects characteristic of CF. Here we review recent efforts to elucidate the impact of genetic variation on the ability of the nascent CFTR polypeptide to interact with the proteostatic environment. We also provide an overview of how specific components of this protein network can be modulated to rescue the trafficking and functional defects associated with the F508del variant of CFTR. The identification of novel proteins playing key roles in the processing of CFTR could pave the way for their use as novel therapeutic targets to provide synergistic correction of mutant CFTR for the greater benefit of individuals with CF.

1. Background

The post-genomic era has led to the development of new tools to explore the mechanisms for onset and progression of countless human diseases including Cystic Fibrosis (CF).

Despite the extensive knowledge acquired by the CF community over the past 3 decades, many potential questions remain about the protein-protein interactions that regulate the trafficking and activity of the CF transmembrane conductance regulator (CFTR) protein, a cAMP-regulated chloride channel whose mutations cause the loss of ionic and fluid homeostasis, characteristic of CF, through different mechanisms. Indeed, CFTR mutations have been grouped into six classes [1]. Mutations introducing a premature termination codon are grouped into class I. Class II CFTR mutants fail to properly fold, are recognized as defective at the endoplasmic reticulum (ER), and sent to proteasomal degradation. Class III and IV mutations respectively decrease CFTR channel activity and channel conductance. Class V mutations mainly affect CFTR splicing, and lead to reduced synthesis of normal and mutated CFTR protein. Whereas class VI, comprises mutations that give rise to a functional protein having abnormally short residence time at the apical plasma membrane. CFTR does not operate in isolation in the complex environment of the cell, but rather is constantly acted upon by a dynamic network of interacting components, referred to as the CFTR Functional Landscape [2], that impact its synthesis, folding, stability, trafficking and function. For the F508del variant, an allele carried by more than 70% of patients [3], many of these interactors are deeply connected to a protein folding management system, termed the Proteostasis Network (PN), which is analogous to a 'social network' that determines the fate of this variant polypeptide in each individual with CF [2]. There is also a protein interaction network that regulates the functional properties of the rescued F508del variant. Differences observed between this CF-associated variant network and the WT network, may account, at least in part, for the defective functional properties observed with this common allele [4]. How the nature of the disease-causing mutation contributes to alterations in the protein-protein interaction profile (PIP) of the affected polypeptide will provide insight into how to best manipulate the affected cellular proteome to abrogate the resulting trafficking and/or functional defects.

Here, we review some of the approaches currently ongoing in our labs, namely to globally identify: 1) protein interactors that mediate the biogenesis of multiple CFTR variants (CFTR interactomes), 2) the trafficking machinery and members of the PN which, when manipulated, can rescue the trafficking and functional de-

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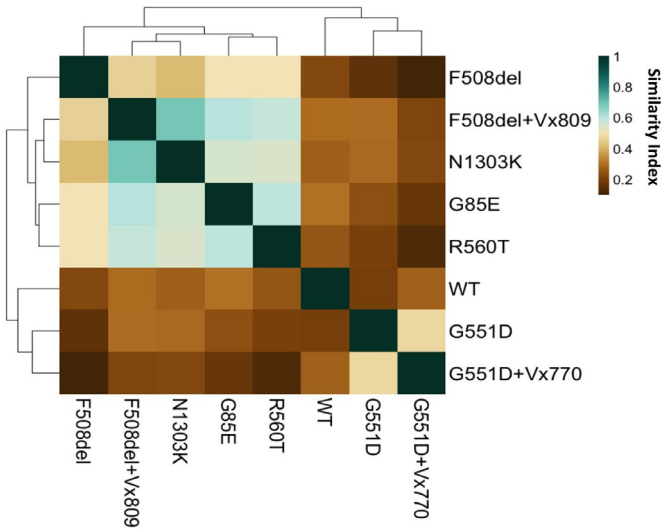

Fig. 1. Heat map of the Jaccard similarity indices calculated from the composition of the respective protein interactomes for the indicated pairwise comparisons of CFTR variants or treatment conditions [5].

fects of F508del-CFTR, and other variants (CFTR secretome) and 3) the proteins that control CFTR function at the cell surface (CFTR functionome).

Altogether, these data provide us with a much more realistic view of the 'CFTR regulome' which will help us make more accurate predictions on the diagnosis, prognosis and therapeutic options for individuals with CF.

1.1. Characterizing the CFTR variant specific interactome

The characterization of proteins interacting with the WT- and F508del-CFTR has allowed us to identify many potential therapeutic targets for correcting the trafficking defect associated with the F508del variant [5–7]. While this approach has provided significant insight into the proteomic components contributing to its defective trafficking, no information exists on the cause of the ER-retention of other class II CFTR variants and whether they share a causal link. Additionally, very little is known about possible differences (as compared to WT-CFTR) in the network of proteins interacting with class III-IV variants and whether the protein network contributes to the functional defect. While there has been some recent progress in developing small molecule correctors, which improve the trafficking of class II variants, and potentiators, which increase the open probability (Po) of class III & IV variants, their precise impact on the CFTR polypeptide remains enigmatic. We used affinity purification mass spectrometry (AP-MS) to address these very questions.

In a first part, we compared the PIP of four class II variants, namely G85E, F508del, R560T and N1303K, which are distributed throughout the CFTR protein, to assess how similar their PIPs were and to attempt, for the first time, to address if they shared commonalities that contributed to their ER retention profile. While the composition of the PIP of these class II variants were similar, exhibiting a Jaccard Similarirty Index $>$ 0.5 (Fig. 1), the affinity of these proteins for the various disease-causing variants differed significantly [5]. Despite these differences, we were able to identify a number of high affinity binding proteins, which, when down regulated by siRNA-mediated silencing, were able to improve the trafficking efficiency of many of the variants studied, with the G85E being the most refractive [5]. While we have characterized the impact of common class II interacting proteins on the trafficking of these variants, more in depth analyses will be required to understand the functional implications of proteins bound to single class II variants. An examination of the impact of the CFTR corrector, Lumacaftor (VX-809), revealed that it significantly shifted the F508del-specific PIP to a more WT-like interactome (Fig. 1) [5]. We were able to confirm that many of the affected proteins could be individually targeted to promote a modest improvement in the amount of cell surface localized F508del-CFTR [5].

While characterizing therapeutic targets for correcting the most common CF-associated variant is critical, many other common disease-associated alleles in the patient population exhibit WT-like trafficking. The ability of these variant polypeptides to traverse the endomembranous compartments of the cell with WT-like efficiency would lead us to hypothesize that they must also exhibit WT-like PIP. We tested this hypothesis using AP-MS to compare the PIP of G551D-CFTR, the 3rd most common clinical variant in the CF population, with that of WT-CFTR [5]. Here, we observed that this class III mutant exhibited a PIP that was more divergent to that of WT than observed for the pairwise comparison between F508del- and WT-CFTR (Fig. 1) [5]. Interestingly, the G551D mutant failed to properly interact with a cohort of related trafficking and cytoskeletal remodelling components involved in modulating sub-plasma membrane (PM) trafficking of cell surface proteins [5]. These data suggest that part of the defect associated with the G551D mutant is its inability to be properly delivered to or retained in the PM, accounting for its reduced channel activity. We also observed that the FDA-approved CFTR potentiator, Ivacaftor (VX-770), is able to restore a more WT-like PIP to the G551D variant (Fig. 1), including increasing the binding affinity to component of the afore mentioned sub-PM trafficking network [5].

1.2. CFTR secretome: protein interaction network from trafficking-based screen

It has been known for some time that the F508del mutant leads to CFTR protein misfolding and recognition by the ER quality control (ERQC) machinery, which targets this CF-causing mutant for premature degradation via the ubiquitin-proteasomal pathway, thus preventing it from trafficking to the cell surface. However, a global view of the mechanisms and pathways involved in this ER retention and degradation are still missing.

We have used a functional genomics approach (high-content siRNA screen) to provide a mechanistic characterization of the proteomic components contributing to the F508del-CFTR trafficking defect. To accomplish this task, we developed a high-throughput microscopy assay to identify factors that could be targeted to restore F508del-CFTR at the cell surface of human bronchial epithelial cells [8]. This assay and pipeline were applied to screen a library of siRNAs targeting the druggable genome.

The primary screen identified $>$200 genes that, when silenced, rescued F508del-CFTR trafficking. A subset of these hits were validated by secondary siRNAs, therefore establishing them as contributing to the ER retention of F508del-CFTR. To provide relevance and specificity of these trafficking regulators as potential drug targets, we performed: *i)* pathway classification (GO terms); *ii)* overlap analysis with the general secretome [9]; *iii)* functional characterization according to ERQC checkpoints [10,11] and *iv)* overlap analysis with existing CFTR interactomes which however consisted in a very reduced number of genes [6,12,13]. Results from these analyses pointed to a complex involvement of several cellular functions in the regulation of the CF pathophysiology and led to the identification of novel potential drug targets, which target the causal molecular defect of this disease. These data, together with expression data in human airways, provide hit relevance for CF and those not-overlapping with the general secretome help to define the specificity of the identified F508del-CFTR trafficking regulators.

1.3. CFTR functionome: protein interaction network from function-based screen

In addition to components of the CFTR secretome, whose modulation can lead to increased trafficking of CFTR mutants, other factors can regulate CFTR function at the cell surface, including proteins involved in peripheral checkpoints, CFTR endocytosis and recycling. To identify these proteins and understand their contributions to CFTR trafficking and activity, we combined an siRNA screening approach, similar to the one utilized to unravel the CFTR secretome, with a functional readout, which is based on monitoring the fluorescence of the halide-sensitive Yellow Fluorescent Protein (HS-YFP) [14].

The first siRNA library was based on a hypothesis-driven approach, which targeted proteins predicted to play a key role in F508del-CFTR processing and degradation, as identified in previous interactome and gene expression profile analyses [15]. Here we identified RNF5/RMA1, an E3-ubiquitin ligase acting early in CFTR biosynthesis [16,17], as an important factor for rescuing F508del-CFTR function [15]. The physiological relevance of RNF5/RMA1 silencing for improving the CF phenotype was confirmed in F508del/F508del mice, where we observed increased CFTR activity and attenuation of the intestinal malabsorption phenotype typically seen in these animals [15]. These data support the rationale for the development of an RNF5/RMA1 inhibitor as a potential CF therapeutic molecule [18].

We identified 37 additional siRNA targets whose silencing restored F508del-CFTR function from the druggable genome siRNA library composed of 6650 therapeutically validated gene targets and designed to specifically support drug screening programs [15]. They included transcription factors (TF) and degradation components [19], including UBA2 and UBE2I, ubiquitin ligases of the sumoylation pathway [20,21] and UBXD1, a Valosin-Containing Protein (VCP)/p97-interacting protein involved in ER-associated degradation [22]. While additional studies will be required to unravel the mechanism of action through which the suppression of TF results in increased CFTR function, we can hypothesize that they act by reprogramming the cellular PN to generate an environment that is permissive for CFTR trafficking and function [23,24].

The most therapeutically relevant target identified in our screening effort is FAU, an uncharacterized fusion protein, consisting of the ubiquitin-like protein FUBI at its N-terminus and the ribosomal protein S30 at its C-terminus. Interestingly, the silencing of FAU increased PM-localized F508del-CFTR in cultured and primary bronchial epithelial cells without affecting WT-CFTR [19]. While we hypothesize that it is part of the ERQC network, modulating the processing of F508del-CFTR, the precise mechanism by which FAU specifically targets mutant CFTR, leading to its degradation remains enigmatic [19]. The identification of FAU as a component of the ERQC machinery that specifically recognizes mutant CFTR, may unravel novel mechanisms underlying protein synthesis and processing.

2. Future directions

The identification of the *CFTR* gene [25–27] brought the promise of finding an effective treatment for CF patients, yet it took more than 20 years before the first CFTR-modulator therapy was approved by the FDA [28]. While the approval of Kalydeco™ and Symdeko™/Symkevi™ provides clinical benefit to many patients, the heterogeneity of their responses and the existence of patients refractory to these therapeutics supports the need for continued scientific exploration into the underlying cause leading to the heterogeneity of the CF phenotype. This includes identifying CFTR interacting proteins [5] and elucidating how they can be manipulated by functional genomic approaches to rescue the trafficking [8] and functional [15,19] defects characteristic of CF.

3. Summary

Here we have reviewed some of our recent efforts to elucidate the impact of genetic variation on the ability of the nascent CFTR polypeptide to interact with the extent proteostatic environment, thereby highlighting both the similarities and differences that contribute to the various CF phenotypes. We also provide an overview of how some of these PN components can be modulated to rescue the trafficking and functional defects associated with the F508del variant of CFTR, the most common CF-associated mutation. How these PN changes impact other CFTR mutants and how they alter the various CFTR interactomes to provide benefits for CF patients will allow us to develop more individualized therapeutic options. Indeed, these prospective drug targets can potentially be manipulated alone or in combination with existing CF therapeutics to provide synergistic correction of mutant CFTR and thus to improve the longevity and quality of life of all CF patients.

Declaration of Competing Interest

The authors declare no conflict of interest.

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