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Review

Hallmarks of therapeutic management of the cystic fibrosis functional landscape

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Abstract

The cystic fibrosis (CF) transmembrane conductance regulator (CFTR) protein does not operate in isolation, rather in a dynamic network of interacting components that impact its synthesis, folding, stability, intracellular location and function, referred to herein as the 'CFTR Functional Landscape (CFFL)'. For the prominent F508del mutation, many of these interactors are deeply connected to a protein fold management system, the proteostasis network (PN). However, CF encompasses an additional 2000 CFTR variants distributed along its entire coding sequence (referred to as CFTR2), and each variant contributes a differential liability to PN management of CFTR and to a protein 'social network' (SN) that directs the probability of the (patho)physiologic events that impact ion transport in each cell, tissue and patient in health and disease. Recognition of the importance of the PN and SN in driving the unique patient CFFL leading to disease highlights the importance of precision medicine in therapeutic management of disease progression. We take the view herein that it is not CFTR, rather the PN/SN, and their impact on the CFFL, that are the key physiologic forces driving onset and clinical progression of CF. We posit that a deep understanding of each patients PN/SN gained by merging genomic, proteomic (mass spectrometry (MS)), and high-content microscopy (HCM) technologies in the context of novel network learning algorithms will lead to a paradigm shift in CF clinical management. This should allow for generation of new classes of patient specific PN/SN directed therapeutics for personalized management of the CFFL in the clinic.

Keywords: Cystic fibrosis; Protein folding; Chaperones; Proteostasis; System biology; Bioinformatics; High-content microscopy; High-throughput screening; Mass spectrometry; Genomics; Proteomics; Epigenetics; Epiproteomics

Contents

Abbreviations: CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; CFTR2, CFFL cystic fibrosis functional landscape; SN, social network; ER, endoplasmic reticulum; MS, mass spectrometry; HCM, high-content microscopy; HTS, high-throughput screening; PN, proteostasis network; UPS, ubiquitin–proteasome system.

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1. Introduction: CFTR is not alone

We are all a product of an evolutionary driven mutational program responsible for survival in response to the environment-called natural selection [1]. We are, therefore, fundamentally mutant by design. This tells us that all biology is designed to work with variants to drive survival and fitness in the playground of life. While cystic fibrosis (CF) is triggered by variations of the 'normal' wild-type CF transmembrane conductance regulator (CFTR) genomic sequence, CF is largely a consequence of a poorly understood cascade of folding and protein interaction challenges events that make the CFTR protein generated by each inherited variant genotype 'step outside' its normal functional routine [2–5], missteps that drive disease onset and progression (Pankow et al. (2015), in press, Nature).

To fully understand CF pathology, solve clinical enigmas (e.g., liabilities associated with each variant and the probability of progression of disease along a particular clinical tract) and, importantly, to efficiently treat the personalized form of the disease found in each individual patient, it is critical to remember that like any protein in the cell, CFTR protein does not operate in isolation. Rather, it works in a dynamic network of components that impact its synthesis, folding, stability, intracellular location and function. These are often unique in their levels and activities in each individual person in response to the inherited genome reflecting past, present and impending future environment(s) for each individual/ patient. These interactions comprise what we refer to as the CF 'Social Network (SN)'. In the past, some of these SN interactors have been referred to as 'CF modifier genes', although their functional significance for the most part remains elusive. The SN of wild-type CFTR, the prominent F508del mutation, and the many other 2000 or so variants identified to date contributing to clinical disease (referred to as the 'CFTR2' cohort [6,7]) are deeply connected to an extensive protein fold management system, the Proteostasis Network (PN) [3,4,8–14]. This PN-coupled SN, herein referred to as the CFTR Functional Landscape (CFFL), is optimized by biology so that a given cell, tissue and individual display a particular functional genotype to phenotype relationship that plays out in health for wild-type CFTR, or as 'variations on a theme' of CF disease for each CFTR variant in each patient. In essence, each CFTR variant can be viewed as an 'outcast' that causes the changes in the highly evolved PN and SN interaction strategies that manage the final physiology of ion transport and divergence from healthy tissue, thus ultimately causing disease state. Such PN and SN 'outcasting' (i.e., divergence from the norm) triggers the multiplicity of both common and variable changes in interactions resulting in variable CF manifestations and disease progression, which are unique to each patient and that contribute to the current concepts of 'personalized' or 'precision' care in the clinic. In support of this view, we have recently shown that the F508del CFTR variant will generate a surprisingly large number of new PN and SN interactions that that collectively drive CFFL pathophysiology and, remarkably, can be largely corrected by the appropriate therapeutic management (Pankow et al. (2015), in press, Nature).

2. Getting new therapeutics by understanding the CFFL

It is now recognized that proteins, particularly membrane-spanning proteins such as (normal) CFTR, face major energetic challenges to fold in the context of the lipid bilayer as well as divergent cytosolic and compartment specific environments [15,16]. Moreover, most proteins are highly dynamic and conformationally challenged, often being biologically 'disordered' even in the healthy setting. These states are further perturbed by the genotype sequence variants initiating disease in a particular cell, tissue or patient environment. Therefore, any attempt to understand disease from in vitro or in vivo heterologous cellular models of function that do not normally express this protein will have limited success, albeit potentially targeting evolutionarily conserved features of CFTR functional (partial) responses to the PN and/or SN management. In this view, structural snapshots of '(mis)folded' states derived from biophysical approaches such as X-ray crystallography structures (the presumed holy grail of contemporary biochemistry) and/or computational 'modeling' approaches based on homologous proteins, necessarily provide limited insight into CF therapeutics in the biological setting. Clearly these approaches fail to grasp what is necessarily the more physiologically relevant dynamic PN and SN that contribute to the local physiologic environment of CFTR in each cell, tissue and patient environment in a particular time-frame ranging from early development to aging [17,18].

A rational approach for defining the physiologic source of an unfavorable CFFL stemming from a CF-causing CFTR variant is to understand the biological PN and SN 'disconnections' that are the 'root' cause of the disease [3] (Pankow et al. (2015), in press, Nature). Emerging insights suggest that these links are mismanaged through multiple mechanisms, leading to both the loss and/or gain of aberrant protein interactions [2–4,7,19–21] (Pankow et al. (2015), in press, Nature). Protein networks normally work together as a highly coordinated 'team effort' using transient, sequential pathways that are unique to