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Cystic fibrosis: Exploiting its genetic basis in the hunt for new therapies

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ABSTRACT

Cystic fibrosis (CF) is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), an anion channel expressed in epithelial cells throughout the body. In the lungs, absence or dysfunction of CFTR results in altered epithelial salt and water transport eventuating in impaired mucociliary clearance, chronic infection and inflammation, and tissue damage. CF lung disease is the major cause of morbidity and mortality in CF despite the many therapies aimed at reducing it. However, recent technological advances combined with two decades of research driven by the discovery of the CFTR gene have resulted in the development and clinical testing of novel therapies aimed at the principal underlying defect in CF, thereby ushering in a new age of therapy for CF.

Contents

1. Introduction

Cystic fibrosis (CF) is a genetic disease of abnormal ion transport. Specifically, abnormalities in the expression and function of the cystic fibrosis transmembrane conductance regulator (CFTR) result in abnormal salt and water transport across epithelial surfaces in the gastrointestinal and hepato-biliary systems, respiratory tract, reproductive system and sweat glands. With the exception of the sweat glands, abnormal salt and water transport eventuate in end-organ damage causing significant morbidity and severely shortening life span. Currently available therapies for CF such as supplemental pancreatic enzymes, antibiotics, mucus thinners, and non-specific anti-inflammatory agents address the consequences of CFTR deficiency rather than the underlying cause. However, decades of research have culminated in the recent testing of therapies that address the basic defect and hold promise for significant clinical benefit. This review attempts to place these recent discoveries in historical context, highlighting how research into the function of CFTR based on the knowledge of the gene prepared the CF community to harness technological advances for the benefit of all CF patients.

2. History of cystic fibrosis

2.1. Determining the underlying defect

The first description of CF as a pathological entity in the United States was published in 1938 by Dorothy Andersen, M.D., a pathologist at The Babies & Children's Hospital of Columbia University in New York City. Her paper entitled "Cystic fibrosis of the pancreas and its relation to celiac disease" firmly established cystic fibrosis of the pancreas as a diagnosis separate and apart from celiac disease (Andersen, 1938). It was not until more than a decade later, however, that the connection was made between salt transport and cystic fibrosis of the pancreas. In 1951, Kessler and Andersen reported on 12

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children admitted to Babies' Hospital with heat prostration who were in relatively good health before a heat wave and who presented acutely with vomiting and signs of shock without evidence of infection. All of these children, except for one who died, responded quickly to rehydration (Kessler & Andersen, 1951). In patients for whom laboratory data were available, serum electrolyte analyses showed low Cl^- and high HCO_3^- concentrations that were reversed with therapy. These findings supported an etiological hypothesis that "fibrocystic disease is associated with widespread abnormality of epithelial glands (Kessler & Andersen, 1951)."

Following these observations, Paul di Sant'Agnese, M.D., also at Columbia University, prospectively studied sweat electrolyte levels in 43 CF patients and 50 control patients. His results demonstrated that Na^+, K^+ and Cl^- all were elevated in the sweat of CF patients, with sweat Na^+ and Cl^- levels being markedly elevated (Di Sant'Agnese et al., 1953). The authors also demonstrated that the elevated sweat Na^+ and Cl^- levels were not the secondary result of pancreatic dysfunction, pulmonary disease, adrenal dysfunction or renal disease, and concluded that the increased susceptibility to dehydration in CF was due to increased salt loss from sweat glands. These findings led directly to the development of the sweat test as a diagnostic test for CF (Gibson & Cooke, 1959).

By the early 1980s, observations in the sweat gland, pancreas and respiratory tract began to suggest that CF was, at its essence, a disease of altered anion transport. Multiple studies confirmed the findings of di Sant'Agnese that sweat electrolyte concentrations were abnormal in CF. In separate studies using different techniques, Quinton (P. M. Quinton & Bijman, 1983) and later Fromter (Bijman & Fromter, 1986) concluded that CF sweat glands had decreased ductal Cl^- permeability and reduced secretion in response to adrenergic stimulation. Similarly, studies of pancreatic HCO_3^- secretion in CF patients concluded that abnormal pancreatic secretion in CF could be attributed at least in part to altered Cl^- secretion (Kopelman et al., 1988). Knowles et al. at the University of North Carolina at Chapel Hill reported that the electrical potential across the nasal epithelia (the nasal potential difference or NPD) of CF patients was more electronegative than control patients (Knowles et al., 1981) and that the apical plasma membrane of nasal epithelial cells in CF patients with CF was impermeable to Cl^- (Knowles et al., 1983). It seemed, then, that the basic defect in three different organ systems could be attributed to altered Cl^- permeability.

2.2. Finding the CF gene

Armed with the knowledge that CF was a disease of altered Cl^- transport, researchers began to search for the affected gene. In 1985, two laboratories using different markers for linkage analysis localized the gene to the long arm of chromosome 7 (Knowlton et al., 1985; Wainwright et al., 1985). In 1989, in a collaborative international effort, Tsui, Riordan, Collins and colleagues discovered the gene responsible for CF (Kerem et al., 1989) and found that in the majority of CF patients the gene was missing three nucleotides that resulted in the in-frame deletion of a phenylalanine residue at position 508 of the polypeptide chain (ΔF508) (Riordan et al., 1989). They designated the protein the cystic fibrosis transmembrane conductance regulator, or CFTR (Riordan et al., 1989). In doing so, the group recognized that if CFTR was not itself a chloride ion channel, then the protein would almost certainly function as a regulator of Cl^- channel activity.

3. The cystic fibrosis transmembrane conductance regulator protein

3.1. Normal CFTR

Even before the CFTR gene was cloned, it was known that cAMP-stimulated Cl^- secretion was defective in CF epithelial cells (Frizzell et al., 1986). Shortly after the CFTR gene was identified, data emerged that the defective cAMP-mediated Cl^- secretion could be corrected by expression of normal CFTR, but not by expression of ΔF508 CFTR. These data supported the hypothesis that CFTR was a Cl^- channel, but still left open the possibility that CFTR was functioning as a positive regulator of another Cl^- channel (Rich et al., 1990), (Drumm et al., 1990). In 1991, Anderson et al. at the University of Iowa expressed recombinant CFTR in three different cell lines and conferred on those cells a cAMP-activated Cl^- conductance that was not found in cells expressing ΔF508 CFTR (Anderson et al., 1991c). These results were independently confirmed in other cell lines (Rommens et al., 1991), (Kartner et al., 1991). The demonstration that mutating specific amino acids in CFTR altered the anion selectivity of the ion permeation pathway conferred on cells in which CFTR was heterologously expressed also strongly suggested that CFTR was a Cl^- channel (Anderson et al., 1991b). Finally, Bear et al. purified the CFTR protein, expressed it in isolated planar lipid bilayers, and demonstrated that it had ion permeation and gating properties identical to those of CFTR heterologously expressed in cell culture (Bear et al., 1992).

When studied by standard electrophysiological techniques in either native or heterologous systems, CFTR has a characteristic biophysical profile. It is an anion-selective channel with a single channel Cl^- conductance of 6–10 picosiemens (pS) in ~120 mM Cl^- and a permeability selectivity sequence $Br^- \geq Cl^- > I^- > F^-$ (P. M. Quinton, 1999; Sheppard & Welsh, 1999). CFTR can also conduct HCO_3^- (Poulsen et al., 1994; J. J. Smith & Welsh, 1992). When studied by patch clamp electrophysiology in symmetrical Cl^--containing solutions, CFTR channels demonstrate a linear current–voltage relationship (Anderson et al., 1991c). The opening of the anion permeation pathway in CFTR requires phosphorylation of the channel, particularly by cAMP-dependent protein kinase A (Cheng et al., 1991), as well as the presence of ATP (Anderson et al., 1991a).

CFTR is a unique member of the ATP-binding cassette family of transporters (ABC transporters), which ordinarily use energy from ATP hydrolysis to pump substrates actively across cellular membranes (David C. Gadsby et al., 2006). CFTR has seven domains: cytoplasmic amino and carboxyl termini, two membrane-spanning domains that each contain six membrane-spanning segments, two nucleotide binding domains (NBD1 and NBD2) and an R, or regulatory, domain (Riordan et al., 1989) (Fig. 1). Although the crystal structure of NBD1 has been elucidated (Lewis et al., 2005), a high-resolution structure of full-length CFTR has not yet been determined. However, homology modeling based on crystal structures of bacterial ABC transporters has provided insights about CFTR's possible three-dimensional structure in cell membranes (Serohijos et al., 2008). Nonetheless, functional studies have revealed how each domain plays a role in the function or regulation of the channel. The putative twelve transmembrane helices provide the anion permeation pathway and contain the gate that controls transmembrane anion flux (reviewed in Linsdell, 2006). The two nucleotide binding domains of CFTR bind and/or hydrolyze ATP to modulate channel activity in a manner that is not yet completely determined, but likely involves dimerization of the two NBDs (Vergani et al., 2005). Nucleotide binding and/or hydrolysis induces conformational changes of the NBDs that are somehow communicated to the channel gate in the transmembrane domain resulting in its opening and closing. This communication may be mediated by extensions of the transmembrane helices that interact with the NBDs. The R-domain of CFTR, unique among ABC transporter family members, is rich in consensus phosphorylation sites, mainly for protein kinases A and C (D. C. Gadsby & Nairn, 1999). However, other kinases can also phosphorylate CFTR (Picciotto et al., 1992). Phosphorylation of CFTR is necessary for its activation (Anderson et al., 1991a), and CFTR channels are deactivated upon dephosphorylation carried out by protein phosphatases (Berger et al., 1993; Reddy & Quinton, 1996). The amino and carboxyl terminal regions of CFTR have specific amino acid residues that allow it to bind to intracellular proteins (W. B. Guggino & Banks-Schlegel, 2004). For