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From basic science to the clinic: where are we and what is still missing

M.D. Amaral

University of Lisboa, Faculty of Sciences, BioFIG-Centre for Biodiversity, Functional and Integrative Genomics and Department of Genetics, National Institute of Health, Lisboa, Portugal

The main cause of cystic fibrosis (CF), the most common life-shortening recessive genetic disease in Caucasians, is a deletion of 3 nucleotides resulting in loss of phenylalanine at position 508 of the CF transmembrane conductance regulator (CFTR) protein. This mutation, termed F508del and accounting for ~ 70% of CF chromosomes worldwide, occurs in ~ 90% of CF patients in at least one allele and is associated with a severe clinical phenotype. However, to date ~ 1,600 *CFTR* gene variants were identified in association with Cystic Fibrosis[1].

In normal individuals, CFTR protein is a cAMP-activated chloride (Cl^-) channel expressed at the apical membrane of most surface epithelial cells, like the airways and the gastrointestinal tract, but also in cells lining the ducts of several glands, like the sweat gland, the pancreas and the submucosal glands. Due to the absence/impairment of functional CFTR in epithelia of CF patients very little or no CFTR-mediated Cl^- transport is generally observed.

Although in principle, restoration of CFTR-mediated Cl^- transport should clinically improve CF, the very high number of *CFTR* gene alterations make this a hard task. Accordingly, most research efforts are thus aimed at understanding the molecular and cellular defects associated with the F508del mutation, so as to identify and correct the cellular defect that it brings about.

The major defect caused by F508del on CFTR protein is at the level of its intracellular localization. Indeed, the F508del-CFTR protein fails to traffic to the plasma membrane, being mostly retained and degraded at the level of the endoplasmic reticulum (ER). Retention at the ER results from failure in the acquisition of a native (folded) conformation by the mutant protein, being the abnormal misfolded form recognized by the ER quality control (ERQC) which sends it to degradation via the ubiquitin-(Ub)proteasomal pathway (UPP) (reviewed in Amaral[2]). Overcoming this intracellular retention of F508del-CFTR would be of great therapeutic value, since this mutant is still functional, albeit only ~ 10-20% of normal CFTR. To correct for these distinct defects associated with F508del-CFTR, two kinds of recent and promising therapeutic small molecules are being searched for, namely: 1) those rescuing the trafficking defect, which are termed "correctors"; and 2) those overcoming/enhancing the defective Cl^- channel activity, called "potentiators"[3].

On the other hand, to rescue the very high number of CFTR gene variants so far found in CF patients, these have been classically grouped into functional classes, which potentially can be corrected through the same restoring strategy applied to F508del-CFTR or by a different one, by an approach coined as "mutation-specific therapy"[3]. This strategy thus relies on functional classification of CFTR mutations into five main categories[3], namely: i) class I mutations, which prevent protein production, often being nonsense mutations (e.g., R1162X), *i.e*, generating premature stop codons that often lead to the degradation of the respective mRNAs; ii) class II mutations (including F508del) causing intracellular retention of the mutant protein and subsequent degradation thus preventing these mutant proteins from reaching the cell surface; iii) class III mutants (e.g., G551D) that allow protein to traffic to the cell surface, but cause impairment in the CFTR channel opening (gating); iv) class IV mutants which cause substantial reduction in the flow of Cl^- ions that permeate through the CFTR channel (e.g., R334W); and v) class V mutants (including alternative splicing mutants, e.g., 3272-26A > G) which still allow for the synthesis of some normal CFTR mRNA and protein, but at dramatically reduced levels due

to the major production of aberrantly spliced CFTR mRNA. Class IV and class V mutants, which still allow residual CFTR-mediated Cl^- transport, are generally associated with milder clinical phenotypes.

Several therapeutic strategies adopting this 'mutation-specific' approach to correct the basic defect in CF are currently under experimental testing or already progressed to the clinical setting (reviewed in Amaral[3]). Examples include:

- Class I: aminoglycoside antibiotics have been described to suppress premature termination codons by allowing the incorporation of an amino acid, thus permitting protein synthesis to continue until its normal termination. An example is the clinical trial carried out in CF patients with stop mutations (namely, W1282X) through gentamicin-induced correction, although a more recent clinical trial has failed to demonstrate efficacy, suggesting that this correction may be mutation-specific.
- Class II: Chemical, molecular or pharmacological chaperones, generally called "correctors" were described to stabilize protein structure and promote folding, allowing cell surface expression of trafficking mutants. These small molecules are expected to mimic the action of the molecular chaperones, which within the living cell help "client" proteins to acquire their native conformation (reviewed in Amaral[4]). Already early this year, one corrector compound VX-809 (Vertex Pharmaceuticals Inc, San Diego, CA) went to Phase I of clinical trial for F508del-homozygous patients. More compounds of this type from a number of other companies are expected to hit the clinical setting very soon.
- Class III: CFTR activators such as alkylxanthines (CPX) and the flavonoid genistein were shown to overcome defects in the gating of the CFTR channel, thus acting as "potentiators". F508del-CFTR is also a class III mutant, since membrane localized channels also exhibit impaired gating and thus reduced channel activity (see above). Recently, the US Cystic Fibrosis Foundation announced that VX-770 (also from Vertex Pharmaceuticals Inc, San Diego, CA), a potentiator drug that stimulates the opening of CFTR mutants with a gating defect, showed promising results in an ongoing Phase 2a clinical trial involving CF patients carrying the G551D mutation who took this drug orally.
- Class IV: Compensation for reduced conductance of these mutants can be achieved by increasing the overall cell surface expression of these mutants through promotion of their traffic with corrector compounds or/and through increased stimulation of the existing channels with potentiators (such as VX-770).
- Class V: Splicing factors that promote normal exon inclusion or factors that promote abnormal exon skipping were shown to increase levels of correctly spliced transcripts *in vitro*. While this kind of correction is still a long way from the clinic, potentiators can also be useful for these mutants, since they enhance the activity of normal CFTR which for these mutants is already present at the cell surface.

Still, as mentioned above, application of this strategy to the clinical setting has its caveats, no matter how appealing it is. In fact, despite causing the same consequences at the cell level, a group of CFTR mutations within the same functional class may respond differently to a given compound.

Other CFTR-independent strategies include the so-called "by-passing approaches" which attempt to manipulate other ionic conductances to the benefit of CF. Among the most promising of these are the stimulation of alternative epithelial Cl^- channels, namely the recently identified Ca^{2+}-activated Cl^- channels (CaCCa) and/or the reduction of the Na^+ hyperabsorption that occurs in CF mediated by the epithelial Na^+ channel (ENaC) and which is regulated by CFTR. Examples of current drugs using these approaches include stimulation of ATP-activated purinergic receptors by synthetic nucleotides which are more stable than ATP, such as INS365 or by compounds like denufosol. The latter is already in Phase III of clinical trial and has the advantage of stimulating both CaCC channels and also of inhibiting ENaC.

To date, gene therapy has failed to demonstrate a clinical benefit for CF after repeated administration. Notwithstanding, a lot was gained from the pre-clinical and clinical studies that were so far performed and the upcoming clinical trial by the UK Gene Therapy Consortium has certainly increased our expectations.

Pre-clinical validation of this affluence of novel compounds in terms of their efficacy thus requires additional substantial efforts, so that only the best are actually given to patients. Accordingly, validation directly on human tissues *ex vivo* using for instance rectal biopsies becomes an attractive option to help solving such problems, since these tissues are already commonly collected for the diagnosis and prognosis of CF in several European centres. Moreover, establishing adequate therapy endpoints is indispensable to assess drug efficacy and clinical validation, for which the current diagnosis methods (sweat Cl^- measurements, nasal potential difference or Cl^- secretion assessment in rectal biopsies) may be extremely helpful. With such a concerted action, it may be possible to achieve the ~ 10% normal CFTR activity, generally believed to be sufficient to cure CF[2].

In conclusion, several novel compounds appear as promising leads to develop effective drugs against the basic defect in CF and the first rationale therapies for CF relying on the understanding of the basic defect have started to hit the clinical setting. Based on the current "drug pipeline", these are expected to rise in numbers very soon.

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