Modulation of protein kinases in the regulation of chaperone machinery leading F508del-CFTR fate

Vilardell J[1], Borgo C[1], Cesaro L[1], Gray M[2], Venerando A[1], Salvi M[1]

[1]Department of Biomedical Sciences, University of Padova, Padova, Italy, [2]Epithelial Research Group, Institute for Cell and Molecular Biosciences, University Medical School, Newcastle University, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK (FFC#10/2016 Pilot) [doi.org/chd8]

Key words: HSP27; protein kinase CK2; F508delCFTR; kinase inhibitors; combinatorial therapy

Background/Rationale. Deletion of phenylalanine 508 in CFTR (F508del) is the most common mutation in Cystic fibrosis (CF) patients (70-90%). F508del-CFTR maintains channel activity, but the mutation causes the majority of CFTR protein to be retained in the endoplasmic reticulum and prematurely degraded by the ubiquitin-proteasome system before it reaches the plasma membrane. The combination of lumacaftor-ivacaftor (corrector plus potentiator, Orkambi™) represents the first FDA-approved therapy for CF patients homozygous for F508del-CFTR mutation, and in general a combination of therapy seems to show improved clinical benefits over available monotherapies (1). CK2 has been suggested as a potential target to rescue the membrane restoring autophagy while epigallocatechin gallate, selected for its ability to target the protein kinase CK2, sustains its permanence at the plasma membrane, even if not able alone to rescue F508del-CFTR (2).

Hypothesis and objectives. The main aim of the project was to reveal the molecular mechanism and clarify the role of CK2 in regulating F508delCFTR stability. Indeed CK2 inhibitors should be used in combination with F508delCFTR rescuing molecules, as they would permit an increase stability of the protein but not the maturation of F508del-CFTR to the plasma membrane where it exerts its function. Understanding the precise mechanism(s) of action of this class of compounds is the basis for developing an efficient combinatory therapy.

Essential Methods. We performed in vitro and in cells studies to demonstrate how CK2 can affect F508del stability using transfections, specific CK2 kinase inhibitors and CK2 knockout cells.

Results. We have demonstrated that CK2 inhibition may represent an important tool to reduce the expression of HSP27, a chaperone that specifically recognize F508del leading to its ubiquitylation and degradation (3). Moreover the detailed mechanisms by which HSP27 expression is regulated by CK2 has bees elucidated. However, the direct correlation between CK2-HSP27-F508delCFTR is still lacking and is under study.

Spin-off for research & clinical purposes. These pre-clinical study are important to provide the rationale for the use of CK2 inhibitors in combinatory therapies with correctors or proteostasis regulators for the rescue and stabilization of F508delCFTR at the plasma membrane.

References

1. G. Veit et al., From CFTR biology toward combinatorial pharmacotherapy: expanded classification of cystic fibrosis mutations. Mol Biol Cell 27, 424 (Feb 01, 2016). https://doi.org/10.1091/mbc.E14-04-0935 PMid:26823392 PMCid:PMC4751594

2. D. De Stefano et al., Restoration of CFTR function in patients with cystic fibrosis carrying the F508del-CFTR mutation. Autophagy 10, 2053 (2014). https://doi.org/10.4161/15548627.2014.973737 PMid:25350163 PMCid:PMC4502695

3. A. Ahner, X. Gong, R. A. Frizzell, Cystic fibrosis transmembrane conductance regulator degradation: cross-talk between the ubiquitylation and SUMOylation pathways. FEBS J 280, 4430 (Sep, 2013). https://doi.org/10.1111/febs.12415 PMid:23809253 PMCid:PMC4156008