UNDERSTANDING BY PROTEOMICS THE CELLULAR TRAFFICKING DEFECT OF A DISEASE ASSOCIATED MUTANT PROTEIN

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Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein, like F508del-CFTR, disrupt intracellular trafficking leading to Cystic Fibrosis (CF) disease. Cells expressing F508de-CFTR restore their ability to exhibit forskolin-dependent chloride transport at cell surface after treatment with a number of chemical an pharmacological chaperones known to stabilize proteins in their native conformation. Mutagenesis on arginine-framed tripeptides (AFTs or RXR) motifs, described to be involved in the endoplasmic reticulum (ER) retention/export quality control of many membrane proteins, was another effort to redirect F508del-CFTR to be functional at the cell membrane. The trafficking defect of F508del-CFTR is temperature-sensitive, as incubation at the permissive temperature of 27-30 °C results in partial protein export from the ER to the cell surface where it functions similarly to the wild-type protein.

Although the effects of all these strategies to rescue the defective F508del-CFTR trafficking and function are well documented the corresponding molecular mechanism is not fully elucidated. Proteins involved in the trafficking defect and/or rescue of CFTR mutants are potential CF therapeutic targets; therefore, we sought to identify these proteins.

We have investigated by proteomics whether BHK cells, the popular heterologous model system for examination of CFTR processing and function, underwent differential proteome modulation in response to the type of CFTR, i.e, wild-type-CFTR, F508del-CFTR or F508del-4RK-CFTR, they are expressing and/or under effect of low temperature (LT) incubation (26ºC).

The results indicated that the mutagenic RXR reverted F508del-CFTR activates a particular unfolded protein response (UPR)/ ER stress pathway in BHK cells that might be able to generate a compatible and/or favourable cellular environment for (at least partial) F508del-CFTR rescuing. Most proteins involved are CFTR- and/or 14-3-3-interactors, suggesting a potential role of these proteins in the CFTR trafficking.

Results from LT experiments showed that under 26ºC, BHK cells are metabolically active and may respond to temperature stress with different strategies from those at

37ºC, namely respecting CFTR processing and trafficking. LT expression profiles of several proteins in BHK-F508del cells tend to wt levels (37ºC), which can be indicative of the involvement of those proteins in rescue of CFTR. Many of them are also described as CFTR interactors (e.g. RACK1). The UPR in BHK-F508del cells under LT treatment seems also to be highly modulated, as many proteins involved in this mechanism were found up-regulated in LT conditions (e.g. GRP78). UPR appears to be particularly related to CFTR rescue by the enhancement of the chaperome/folding environment in the ER.

Several proteins identified in both experiments are also associated with CF inflammation and oxidative-stress pathology.

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