A systems biology approach to the correction of Cystic Fibrosis: from building a network of proteostasis regulatory pathways to combinatorial targeting

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Background. Cystic fibrosis (CF) is a frequent and lethal genetic disease caused by mutations associated with the CF transmembrane regulator (CFTR), a chloride channel located in the apical membrane of epithelial cells lining the ducts. In nearly 70% of the CF patients, the mutation involved is a deletion of phenylalanine at position 508 of the protein (DF-CFTR). The mutant protein cannot fold properly leading to its intracellular retention and degradation. Pharmacological screening approaches have identified small molecule "correctors" (which promote a modest level of DF-CFTR arrival at the plasma membrane), some of which are in clinical trials. Unfortunately, their mode of action is not known.

Hypothesis and objectives. We proposed to develop a rational basis for the pharmacological correction of DF-CFTR defects, by characterizing the mechanism of action of correctors, to identify molecular components and pathways involved in the correction and then targeting them by efficient ways.

Methods. We developed a novel bioinformatic method that we termed Fuzzy intersection of transcriptomes (FIT) to identify genes that commonly regulated by a set of drugs. We used this method to identify genes that are modulated by many of the corrector drugs (we analyzed the transcription profiles of 23 corrector drugs) to achieve their corrective action. These genes were then validated experimentally for their action on regulating DF-CFTR proteostasis.

Results. By using the FIT algorithm on the transcriptional profiles of 23 corrector drugs we arrived a set of 621 genes (219 downregulated and 402 upregulated genes) that we termed correction-relevant (CORE) genes. Out of these we selected 108 genes (based on bioinformatic criteria) to validate experimentally. Of these genes we found 47 to be active in regulating DF-CFTR proteostasis. These 47 genes were then arranged into potential signaling pathways based on KEGG pathway and STRING database, resulting in 8 signaling pathways/networks. Of these we further characterized one pathway that had as the central component MLK3. The known upstream and downstream kinases of the MLK3 pathway were validated experimentally to arrive at the MLK3 pathway that controls the proteostasis of DF-CFTR. The mechanism of MLK3 action on DF-CFTR proteostasis was then analyzed and we found that MLK3 pathway controls the endoplasmic reticulum associated degradation of DF-CFTR and also the plasma membrane quality control of DF-CFTR. Additionally, small-molecule mediated inhibition of the MLK3 pathway was observed to potently rescue DF-CFTR and thus suggesting a potential pharmacological approach to the correction of DF-CFTR.

Spin-off for research and clinical purposes. A rational pharmacological basis of correction of DF-CFTR was developed and potential novel, efficient and specific small molecule reagents that correct the basic defect of DF-CFTR was identified.

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