RNF5/RMA1 ubiquitin ligase as a drug target for mutant CFTR rescue

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Background. Cystic fibrosis (CF) is a severe hereditary disease caused by mutations that abolish the function of a membrane protein (named CFTR) needed to transport chloride ions. The most frequent mutation in CF patients is the deletion of phenylalanine 508 (F508del), causing misfolding and premature degradation of the mutant protein. The trafficking defect can be rescued by molecules called correctors, however, the efficacy of known compounds is reduced. By studying proteins that interact with CFTR we identified the ubiquitin ligase RNF5 as a protein of particular interest, since its inhibition can lead to mutant CFTR rescue both in vitro and in vivo using animal models.

Hypothesis and objectives. In vitro RNF5 silencing causes a significant F508del-CFTR rescue additive/synergic with the effect of the investigational drug VX-809. RNF5 loss in F508del-CFTR transgenic animals ameliorated intestinal malabsorption as evidenced by changes in body weight, and reduced fecal excretion of biliary acids, and concomitantly led to an increase in CFTR activity in intestinal epithelial cells. In vitro and in vivo experiments clearly demonstrate that RNF5 could constitute a strong target for the development of novel therapies for CF.

Methods. The project relied on a computational approach, based on homology modelling followed by high-throughput protein-ligand docking to virtually screen large collection of chemical compounds. Biological evaluation of selected compounds was performed by means of biochemical, microfluorimetric and electrophysiological techniques to measure CFTR-mediated ion transport activity. In parallel, we also developed novel assays based on high-content imaging and analysis to monitor the effects of hit compounds on RNF5 downstream targets.

Results. Our project has led to the identification of a drug-like small-molecule, called 2A2, that efficiently inhibits RNF5 ligase activity and rescue F508del-CFTR in human bronchial cells derived from CF patients. In vitro experiments demonstrated that treatment with inh-02 modulates ATG4B and paxillin, both being known RNF5 targets.

Final results and their significance. These studies point to potential use of RNF5 as a novel target for F508del-CFTR. Our results validate RNF5 as a drug target, providing evidences to support its druggability. In addition, the efficacy of the identified compound as mutant CFTR corrector in primary cells demonstrate the biological relevance of this rescue mechanism.

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