PF-429242 alleviates p.Phe508del-CFTR defects

Raphael Santinelli∗1, Julie Guellec1, Nathalie Benz1, Emilie Luczka2, Christelle Coraux2, and Pascal Trouvé1

1Inserm UMR 1078 – Université de Bretagne Occidentale [UBO] – France
2Inserm UMR-S 1250 – Université de Reims - Champagne Ardenne – France

Résumé

Cystic fibrosis (CF) is the most common lethal autosomal recessive disease found in the European population. It is due to mutations in the CF Transmembrane conductance Regulator (cftr) gene coding for the CFTR protein, a chloride and bicarbonate channel. The most common mutation in CF patients is a phenylalanine deletion (p.Phe508del) at the 508 position in the polypeptide sequence. The p.Phe508del-CFTR protein is misfolded and can’t validate the Endoplasmic Reticulum (ER) quality control steps. It is retained in the ER and rapidly degraded, which has an impact on its ability to be transported into the plasmic membrane. The main cause of morbidity and mortality in CF is the lung defect due to infection and inflammation due to a more viscous mucus production. Inflammation, infections and the misfolded protein’s accumulation trigger the Unfolded Protein Response, a normal physiological process aimed to reduce the ER stress. It is regulated by three interconnected pathways: IRE1α, PERK and ATF6. Previous study showed that the activation of ATF6 downregulates CFTR’s expression and we previously showed that siRNA against ATF6 allows CFTR channel-associated activity restoration. We found a pharmacologic compound, PF-429242 (PF), which inhibit this pathway too. We choose to use a molecular approach because it is more appropriate in a therapeutic view. PF is an anti-serine protease specifically inhibiting S1P which is essential for the ATF6’s activation.

Our aim is to study the effect of PF to rescue CFTR’s activity. All results were obtained in CFBE410- cell line, which are human bronchial epithelial cells from a CF patient homozygote for F508-CFTR mutation. MTT test showed that PF is not toxic for our model. We performed immunocytochemistry to localise ATF6 in cells after treatment. Western blots allow us to study the cleavage of SREBP2 and the expression of GRP78, a hallmark UPR protein, under PF treatment. RT-qPCR aimed 84 UPR specific gene expression. PCR array were used to study XBP1, IRE1, PERK, CHOP and CFTR gene expression after PF. Immunoprecipitation allowed us to see qualitatively the total CFTR amount produced in cells. Finally, patch-clamp experiments were performed to evaluate the rescue of p.Phe508del-CFTR activity. We showed that PF is not toxic. ATF6 cellular localisation is modified after treatment, it is absent from the nucleus showing its inactivity. Looking at the cleavage of SREBP2 by Western blot, we showed that PF is also specific of S1P. CFTR mRNA and global protein amount are overexpressed after PF. We also highlighted 10 modulated genes may explain how PF alleviates the p.Phe508del-CFTR defects. Finally we found that PF restores the Cl-channel function of p.Phe508del-CFTR.

Using PF as molecular ATF6 pathway’s inhibitor we demonstrate that ATF6 is a relevant target to rescue the p.Phe508del-CFTR function in CF.

∗Intervenant