

Cystic Fibrosis Therapies through Non-CFTR Anion Channels / Transporters

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Objectives: Identification of SLC26A9/A4 regulator networks and their overlap with CFTR/TMEM16A

Background: The genetic disease cystic fibrosis (CF) is caused by mutations in the CFTR (Cystic Fibrosis Transmembrane Conductance Regulator), a protein that is expressed at the apical membrane of epithelial cells and directly mediating the transport of chloride (Cl^-) and bicarbonate [1]. Absence of functional CFTR causes a severe imbalance in ion and water transport that leads to a variety of negative effects in various organs, with major impact in the airways, being lung disease the main cause of morbidity and mortality in CF patients [2]. Despite that one single mutation -F508del- occurs in ~85 % of CF patients (85%), there are to date more than 2,000 CFTR mutations reported and > 1,000 mutations are considered as ultra-rare, since they occur in 5 or less families worldwide [3]. Although there is significant progress in CFTR modulator therapies, still the majority of CF (~55%) patients are not eligible for these, because they have either these ultra-rare mutations or the so-called 'CFTR unrescuable' (class VII) variants [4]. It is thus unlikely that these patients will ever benefit from the existing therapies or that drug discovery programmes will be established by Pharma for these ultra-rare mutations. Thus, new therapies that correct the $\text{Cl}^-/\text{HCO}_3^-$ imbalance in CF by stimulation of non-CFTR anion channels/transporters to compensate for the absence of functional CFTR are urgently needed [5]. Among the key alternative channels/transporters, are Solute Carriers (SLC26A9; SLC9A4) stand out as strong candidates [6], as they are apically expressed in several epithelia, namely in the colon, pancreas, kidney, testis and lung [7].

SLC26A4/pendrin acts as an electroneutral anion exchanger of several substrates, but the $\text{Cl}^-/\text{HCO}_3^-$ exchange mode is described as the most predominant. Although both pendrin and CFTR have the ability to transport HCO_3^- , it was recently clarified that pendrin has a crucial function in the control of the composition and pH of secreted fluids, while CFTR regulates the rate of liquid secretion [8].

SLC26A9 in contrast, has been proposed to be a constitutively active and CFTR-regulated Cl^- conductance in human bronchial epithelia with minimal HCO_3^- conductance [9] and recent studies identified this Cl^- channel as a modifier and potential therapeutic target in CF [10]. We have shown that SLC26A9 also contributes to Ca^{2+} -activated Cl^- secretion [11], which is mostly mediated by TMEM16A/ANO1. Notably, two SLC26A9 mutations, found to disrupt its plasma membrane traffic (R575W; V486I), were identified in a patient also heterozygote for F508del in the CFTR gene presenting with diffuse idiopathic bronchiectasis, a major hallmark of CF [12]. This suggests that SLC26A9 and CFTR share a functional epithelial role and thus SLC26A9 may serve as an alternative Cl^- channel to compensate for CFTR dysfunction in CF.

Notwithstanding, the physical and functional interaction between SLC26A4/A9 and CFTR in epithelia is not complete understood and even less so their functional interaction with TMEM16A/ANO1.

We have previously generated bronchial epithelial (CFBE) cell line with stable expression of double-tagged SLC26A9 (eGFP-3xHA) and used this cell line to carry out a pilot siRNA screen by using a

microscopy-based trafficking assay with this cell line. A set of genes was found which when knocked-down affect SLC26A9 traffic to the plasma membrane (PM).

Methodology: This BioSys project will use the previously generated bronchial epithelial (CFBE) cell lines with stable inducible (Tet-ON) expression of double-tagged eGFP-3xHA-SLC26A9 via retroviral (pLVX-TRE3G) transfection. These cell lines express in an inducible way: 1) eGFP-3xHA-SLC26A9 alone (parental); or with 2) mCherry-Flag- wt-CFTR or 3) with mCherry-Flag-F508del-CFTR. These cells will be used to further characterize the genes previously found to affect SLC26A9 traffic, namely to determine whether they are direct interactors and also to determine how they affect PM expression and function using Western blotting, immunolocalization, and transepithelial bioelectric studies in Ussing chambers. The eGFP-3xHA-SLC26A4/9 cell lines will also be used to screen the FDA-approved library of drugs, so as to identify those that affect SLC26A4/9 traffic/function.

Similar cell lines will also be produced using the eGFP-3xHA-SLC26A4 construct (already generated).

Further, this project will also explore the physical and functional interactions of SLC26A4/9 with CFTR (normal and mutant) and TMEM16A/ANO1, by measuring the localization and function of these proteins upon overexpression and downregulation of TMEM16A and CFTR (normal and mutant).

Identification of the regulatory network of these two transporters will shed light into our understanding of their physiological function in epithelia and to gain insight into its possible replacement role in CF.

References

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