BK modulation as a therapeutic target for Cystic Fibrosis

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Introduction

Large conductance calcium-activated potassium channels (BK channels) are vital for maintaining proper hydration of the airway surface liquid (ASL) [1]. We have employed *in silico* (Figures 1, 3 & 4) and *in vitro* methods to examine the potential of BK channel modulators to improve ASL hydration in cystic fibrosis (CF).

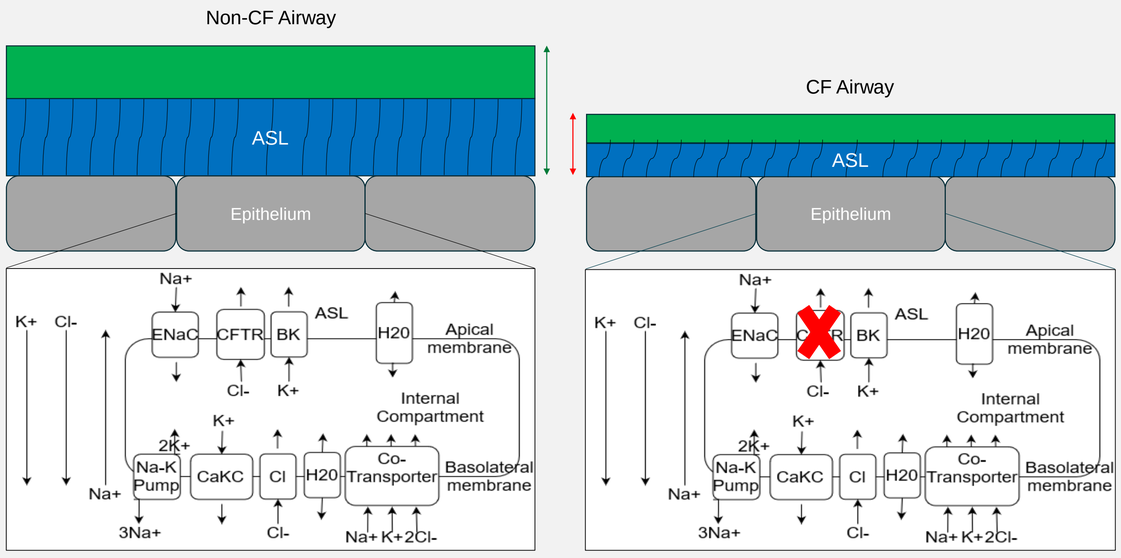

Figure 1: *A diagram highlighting model components for non-CF and CF epithelial cell simulations.*

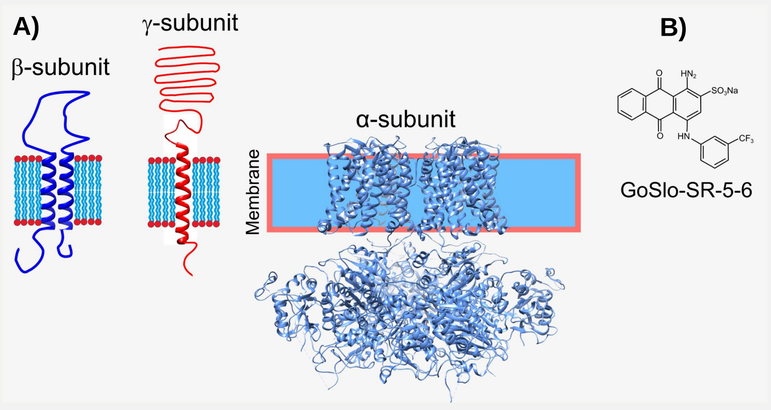

Figure 2. BK channels structure. A) BK channels must contain an α subunit but may also contain auxiliary β or γ subunits. B) Structure of the BK channel activator GoSlo.

BK channels

BK channels are comprised of four α subunits and may co-assemble with β subunits (β1-4) and γ subunits (γ1-4) (Figure 2). The subunit combination present in the airway epithelium is unclear.

Methods

Human bronchial epithelial (HBE) cells, expressing R334W/ΔF508 mutations were grown in air liquid interface (ALI) culture using PneumaCult™-Ex Plus Medium and PneumaCult™-ALI Medium. Cultures were grown in the absence of antifungals, and antibiotics. ASL depth measurements were made using scanning ion conductance microscopy [2]. During measurements an environmental chamber was used to maintain the cultures at 37 °C, 5 % CO_2 and 99% humidity. The BK channel modulator GoSlo-SR-5-6 (GoSlo) was chosen as it is effective for all channel subunit combinations [3].

Simulations

Model Validation

We developed our model as an extension of O'Donoghue et al. (2013) [4] with a variable ASL volume and ion concentrations. We validated our model against the results of Namkung et al. (2009) [5], who measured changes in ASL potassium ion concentrations following application of various blockers (Figure 3)

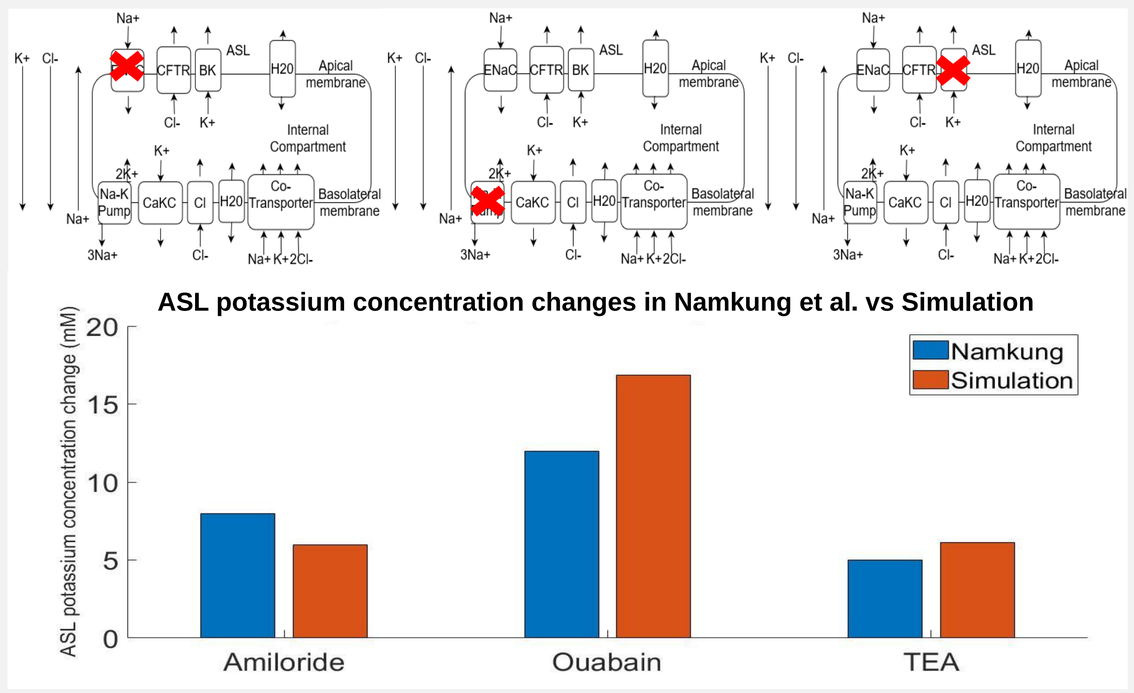

Figure 3: A bar chart comparing the simulation results to that of Namkung et al. (2009) with corresponding schematic diagrams of the simulation carried out in the model

Model Prediction

We used our model to simulate the effect of BK channel stimulation on ASL depth. The model predicts that the BK channel activation can significantly increase ASL depth when there is residual anion permeability was reduced by 75% to replicate the effect of the R334W mutation [7] (Figure 4).

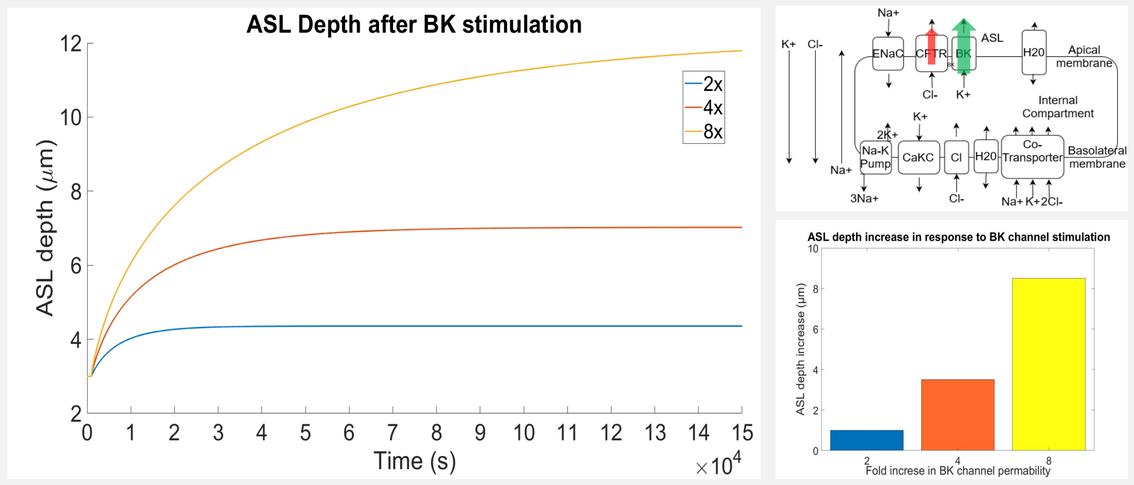

Figure 4: A diagram showing simulation results of BK channel stimulation with corresponding schematic diagrams of the simulation carried out in the model

Experimental Validation

BK activation can increase ASL depth (Figure 5), when R334W CFTR provides residual anion activity. R334W has been reported to be resistant to modulators [6]

Looking across all measurements made going from vehicle (DMSO) to GoSlo shows an increase in the mean ASL depth for seven of eight cultures (Figure 6).

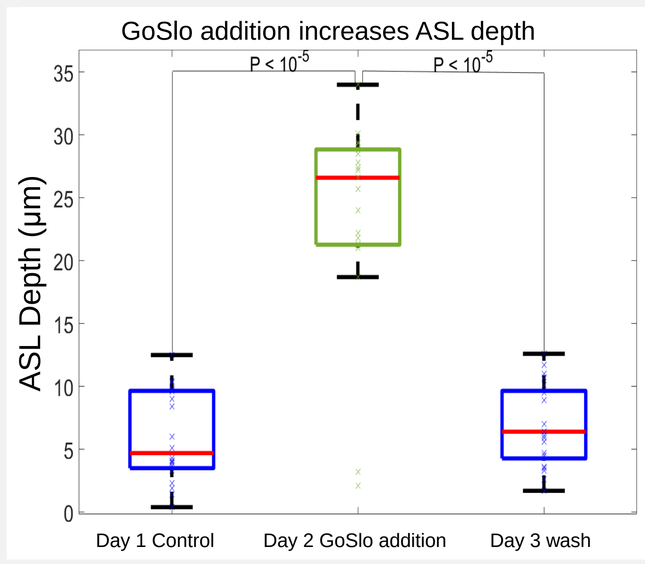

Figure 5: A box and whisker plot showing three days of experimental results applying GoSlo to a R334W/ΔF508 culture

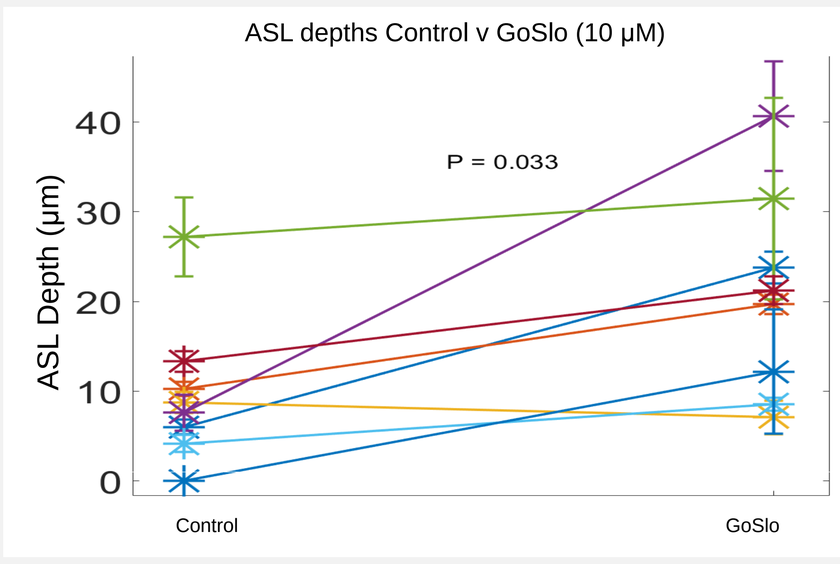

Figure 6: A plot showing the mean ASL depths with standard error bars in DMSO control on the first day and GoSlo on the second

Conclusion

We have shown using *in silico* and *in vitro* methods that BK channels may be a good target for restoring airway surface liquid hydration in CF, provided there is some residual anion channel activity. We have been able to demonstrate this using R334W, which has been reported to be poorly responsive to current CFTR modulators. These findings suggest that BK channel modulation may provide a useful adjunct therapy for CF patients.

Cystic Fibrosis Trust

References:

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