Impact of SHC-1 adaptor protein inhibitors on the plasma membrane abundance of the CFTR channel in epithelial cells

Patrícia Barros[1,2], Mariana F. L. Pereira[1,2], Alexey Tomilov[3], Gino Cortopassi[3], Peter Jordan[1,2], and Paulo Matos[1,2]

[1]*Department of Human Genetics, National Institute of Health Dr. Ricardo Jorge, 1649-016 Lisbon, Portugal.*

[2]*BioISI - Instituto de Biossistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal.*

[3]*Molecular Biosciences, School of Veterinary Medicine, University of California Davis, Davis, California.*

Summary

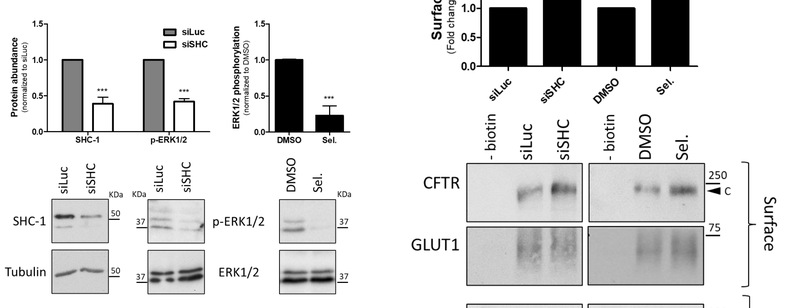
Background : The cystic fibrosis transmembrane conductance regulator (CFTR) is a chloride and bicarbonate channel critical for epithelial ionic homeostasis. CFTR dysfunction, caused by genetic mutations (as in cystic fibrosis, CF) or acquired impairment (e.g., from tobacco smoke or inflammation), disrupts epithelial secretions and contributes to diseases such as chronic obstructive pulmonary disease (COPD). We previously showed in CFBE airway cells that phosphorylation of CFTR at Y512 by spleen tyrosine kinase promotes its internalization from the plasma membrane (PM), a process mediated by the adaptor protein SHC-1. This study aimed to validate this mechanism in additional epithelial models and explore the potential of SHC-1 inhibitors to modulate CFTR PM abundance.

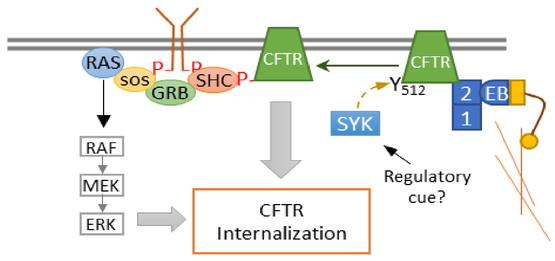
Methods: CFTR trafficking was evaluated in CFBE, 16HBE, and Caco-2 epithelial cells using cell surface biotinylation assays and immunoblotting following RNA interference or treatment with the MEK inhibitor selumetinib, idebenone (IDE), or the novel SHC-1 inhibitor 110#3. Changes in MAPK signaling were assessed via ERK phosphorylation levels. Statistical analysis was performed using ANOVA with post hoc testing.

Results: We confirmed that SHC-1-mediated internalization of CFTR is conserved in 16HBE and Caco-2 cells, both of which endogenously express CFTR. In CFBE cells, IDE and 110#3 increased PM abundance of CFTR in a dose-dependent manner. However, this effect was not CFTR-specific, as both compounds also elevated surface levels of unrelated proteins (GLUT1 and E-cadherin). Importantly, neither IDE nor 110#3 altered PM protein levels or MAPK activity in CFBE or 16HBE airway cells…

Results

Previously…

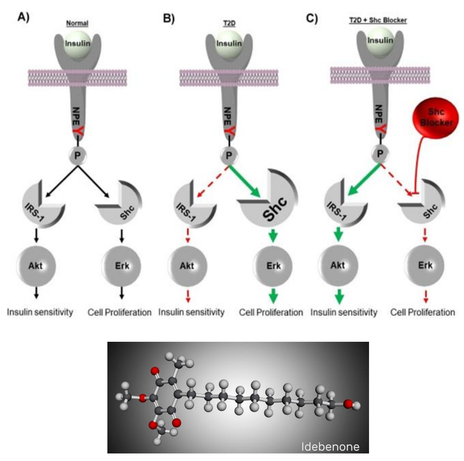




In CFBE airway cells, phosphorylation of CFTR at Y512 by SYK kinase promotes its internalization from the plasma membrane, a process mediated by the adaptor protein SHC-1 that links CFTR internalization to MAPK signaling…

(Barros et al. 2023, doi:10.3390/biom13060949)

Meanwhile…



(A) Upon insulin binding, the insulin receptor (IR) undergoes autophosphorylation and recruits adaptor proteins IRS-1 and SHC-1, which compete for binding to the activated receptor. These adaptors activate distinct signaling pathways: the IRS-1/PI3K/AKT axis, promoting glucose uptake and anabolic metabolism, and the SHC-1/MAPK pathway, associated with cell growth and proliferation.

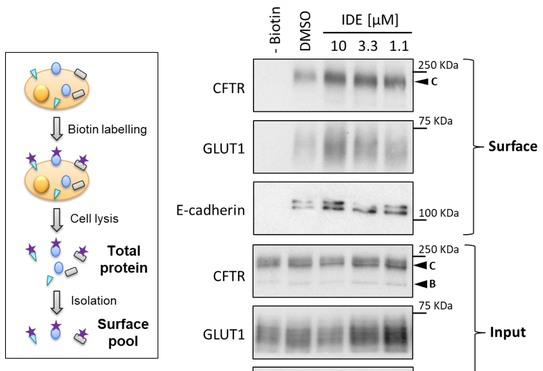
(B) Chronic activation of the SHC-1/MAPK arm contributes to insulin resistance in type 2 diabetes (B).

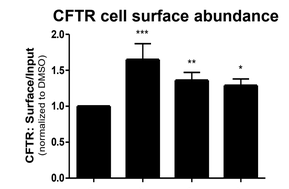
(C) Idebenone (IDE), a synthetic ubiquinone analogue, selectively attenuates this aberrant MAPK signaling, restoring insulin sensitivity *in vitro* and *in vivo*.

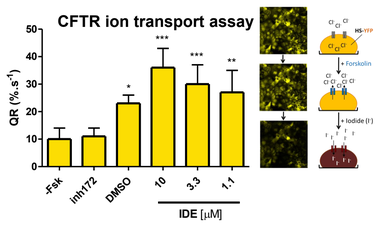
(Jiang, Tomilov et al. 2023, doi:10.1016/j.biopha.2020.110823)

So, can IDE also block CFTR internalization in CFBE airway cells??

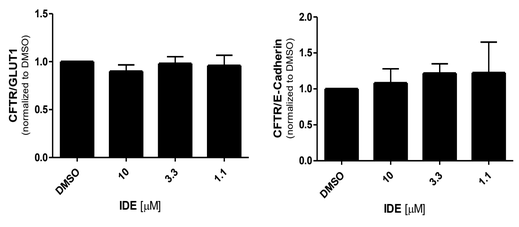
Cell surface protein biotinylation assay



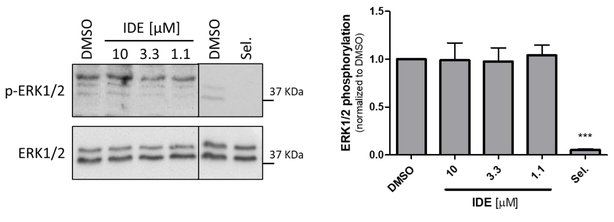




Ratio of cell surface protein abundances



MAPK pathway activity

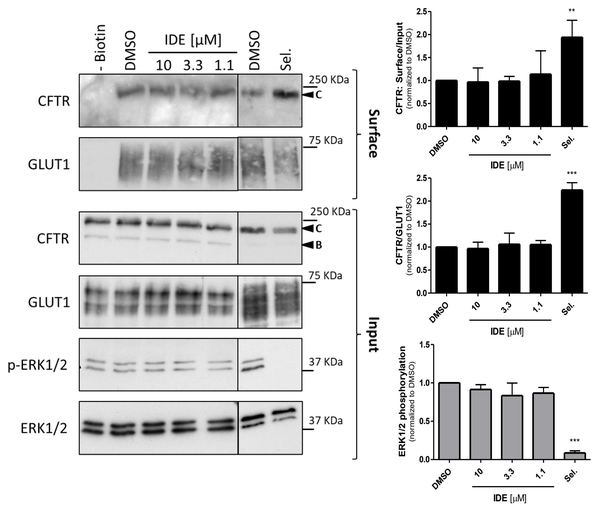


Apparently, yes! But…

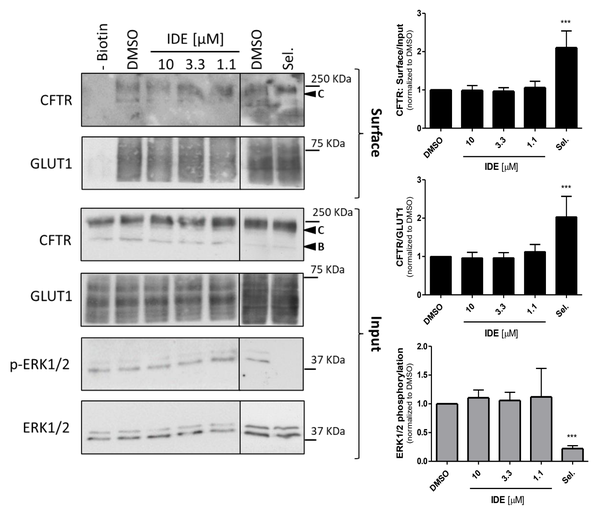
- Increased protein abundance at the cell surface was not CFTR-specific;
- Effect was not dependent on MAPK inhibition?

…is IDE usable??

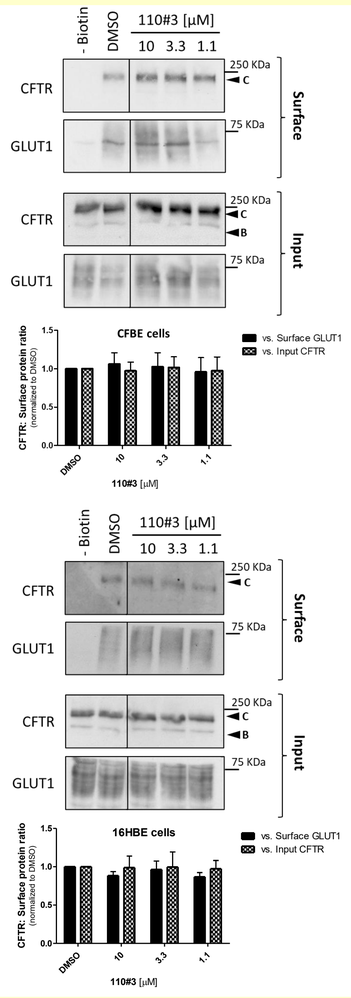
Effect of IDE on 16HBE airway cells with endogenous CFTR expression…



Effect of IDE on Caco-2 colorectal cells with endogenous CFTR expression…



What about using 110#3 – a non-quinone, more specific inhibitor of p-IR/SHC-1 interaction instead of IDE??



Also failed to increase CFTR levels at the cell surface in both CFBE and 16HBE airway cells…!!

Conclusions

- **CFBE cells may be a biased model for studying the effects of pharmacological modulators on CFTR permanence or stability at the plasma membrane (PM), due to their atypical sensitivity to compounds such as IDE;**
- **16HBE and Caco-2 cells, which respond selectively to selumetinib but not to IDE, may represent more reliable models;**
- **Further research is needed to:**
  - ✓ **establish the most predictive models for CFTR surface dynamics;**
  - ✓ **identify selective inhibitors of the SHC-1/pY512-CFTR interaction with potential therapeutic relevance for CF and COPD.**

Work partially supported by UID/04046/2023 - Biosystems and Integrative Sciences Institute Centre grant from FCT, Portugal

Fundação para a Ciência e a Tecnologia