Fenretinide Increases CFTR Functional Expression and Recruitment into Ceramide Microdomains

Asmahan Abu-Arish[1,2], Dusan Garic[3], Radu Pislariu[4], Danuta Radzioch[3,5,6] & John W. Hanrahan[1,2,6]

[1] CF Translational Research Center [2] Dept. Physiology [3] Dept. Human Genetics [5] Dept. Medicine
[6] The Research Institute of the McGill University Health Center, McGill University, Montreal, QC
[4] Laurent Pharmaceuticals, Montreal, QC

Purpose: In this study, we investigate the effects of chronic Fenretinide (Fen) treatment on the plasma membrane distribution of wild-type CFTR (wt-CFTR) under control (Ctr) and stress conditions. We also examine the impact of Fen treatment on CFTR channel function, alone and in the presence of Zinc (Zn) and a CFTR corrector (VX-809, lumacaftor).

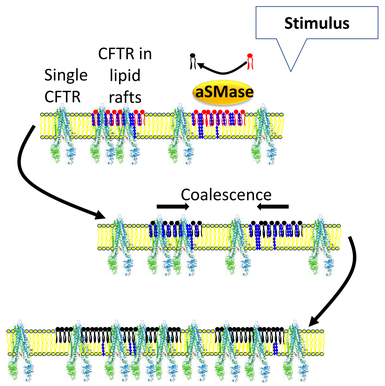
Techniques: We used advanced quantitative fluorescence imaging analyses to study CFTR membrane distribution at the plasma membrane of epithelial cells, and Ussing Chamber for functional studies.

Results: Although Fen did not alter the membrane distribution of wt-CFTR on primary airway epithelial cells under control conditions, it did enhance the partitioning of CFTR into ceramide-rich microdomains during cell stress, suggesting that CFTR recruitment inside these domains may be part of a host defense response that leads to increased pathogen clearance and/or the resolution of inflammation, and helps CFTR stabilization at the cell surface.

Short circuit currents (I_{sc}) mediated by both wt- and F508del-CFTR were increased significantly (30-120%) in a concentration- and time-dependent manner by treatment with Fen and Fen+ Zinc. The effect was enhanced when cells are co-treated with VX-809 (lumacaftor).

Conclusions: The stabilization of CFTR on primary airway epithelial cells depends on membrane lipid composition, especially under stress conditions, suggesting an important link between inflammation and CFTR membrane distribution. The Fen-induced enhancement of functional CFTR expression under cell stress may be due, at least in part, to the rebalancing of ceramide levels in the airway epithelial cells.

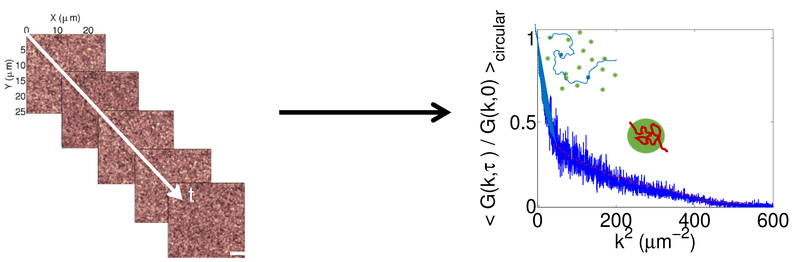
CFTR in lipid microdomains:



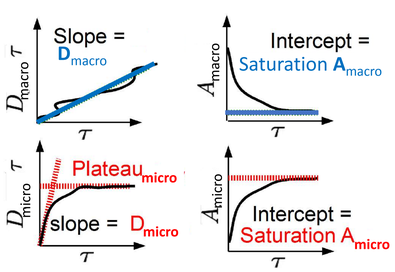
Biochemical & Biophysical studies predicted CFTR existence in lipid rafts

K-space image correlation spectroscopy (kICS):

$$G(k,\tau) = \langle \tilde{\imath}(k,t)\, \tilde{\imath}^{*}(k,t+\tau) \rangle$$



$$\frac{G(k,\tau)}{G(k,0)} = A_{macro} e^{-k^2 D_{macro} \tau} + A_{micro} e^{-k^2 D_{micro} \tau}$$

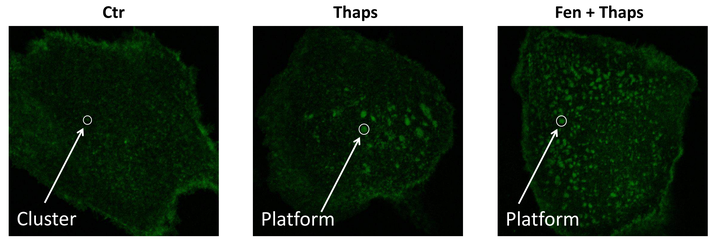


Fit a linear regression to:
The amplitude graphs: A_{macro}, A_{micro} and the mean-square displacement (MSD) graphs: $D_{macro}\tau$, $D_{micro}\tau$, at a given τ range.

$$R = 2\sqrt{Plateau_{micro}}$$

CFTR Recruitment into Ceramide Microdomains

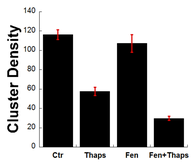
Fenretinide increases CFTR platform formation under stress:



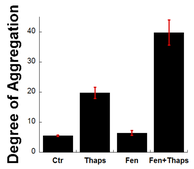
Homogenous distribution of clusters | Platform formation | More platform formation

Confocal imaging shows:
→ Under control (Ctr) conditions, CFTR forms homogenously distributed clusters at the plasma membrane of epithelial cells expressing EGFP-CFTR.
→ Thapsigargin (Thaps) triggers the formation of CFTR-containing platforms, and this is increased with a chronic treatment with fenretinide (Fen).

Fenretinide increases CFTR aggregation under stress:

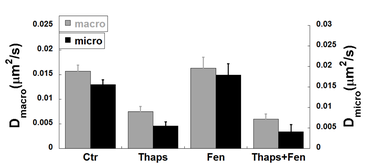


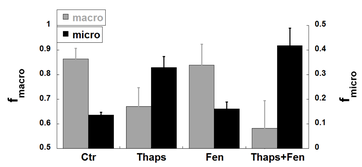
❖ **Using ICS**, Thaps decreases CFTR cluster density (α number of clusters/μm²).
→ Pretreatment with Fen alone doesn't affect CFTR membrane distribution.
→ Exposure to Thaps after a pretreatment with Fen further decreases CFTR cluster density.



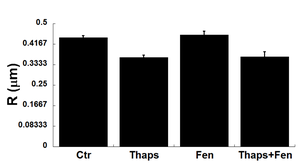
❖ **Using ICS**, Thaps significantly increases CFTR recruitment and aggregation (α size of clusters) inside clusters due to platform formation.
→ Chronic pretreatment with Fen alone doesn't influence CFTR aggregation at the plasma membrane.
→ Exposure to Thaps after a pretreatment with Fen further increases CFTR aggregation inside platforms.

Fenretinide increases CFTR recruitment into platforms under stress:





❖ **Using kICS**, Thaps significantly decreases CFTR mobility at the plasma membrane (D_{macro}) and particularly increases its confinement (D_{micro}) and its recruitment (f_{micro}) inside clusters due to platform formation.
→ Pretreatment with Fen alone does not alter CFTR mobility, confinement or recruitment inside clusters compared to the Ctr conditions.
→ Pretreatment with Fen further increases CFTR confinement and recruitment inside platforms in response to Thaps.

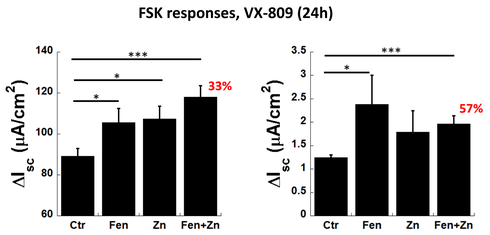


❖ **Using kICS**, Thaps significantly increases CFTR confinement strength inside clusters due to platform formation. (1/R α confinement strength)
→ Pretreatment with Fen does not affect CFTR confinement strength compared to Ctr conditions, either alone or when cells treated with a stressor.

Supported by the Cystic Fibrosis Foundation

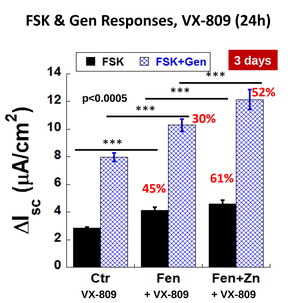
Fenretinide Increases CFTR Function

Fenretinide increases CFTR currents in non-permeabilized airways cells, co-treated with VX-809:



Ussing chambers were used to measure CFTR-dependent current across polarized airway epithelial cells: both fenretinide (Fen) and Zinc (Zn) significantly increase wt- and F508del-CFTR Cl^- conductance compared to controls (Ctr).

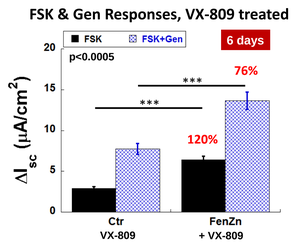
Fenretinide increases F508del-CFTR conductance in permeabilized airways cells, co-treated with VX-809:



To dissect CFTR functional contribution aside from other basolateral channels: polarized airway cells were basally permeabilized for functional studies.

→ Compared to Ctr cells, chronic pretreatment with Fen significantly increases F508del-CFTR function as measured in response to maximal forskolin (FSK) and genistein (Gen) stimulations of CFTR current.
→ A combination of Fen and Zn further increases F508del-CFTR conductance.
→ VX-809 (1uM) was used the last 24h bilaterally

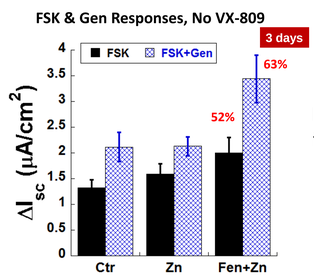
Fen increases F508del-CFTR conductance in a time-dependent manner:



Longer pretreatment with Fen+Zn elicited larger F508del-CFTR functional responses.

VX-809 (1uM) was used the last 24h bilaterally

Fenretinide action is independent of VX-809:



Fenretinide exerts a positive effect on F508del-CFTR functional response independently of VX-809 correction.

Conclusions

❖ Fenretinide increases CFTR recruitment, aggregation and confinement inside lipid microdomains, and enhances the formation of CFTR-containing platforms in response to cellular stress.

❖ Fenretinide increases CFTR (wt & F508del) functional responses in a time-dependent manner, independently of VX-809 correction mechanisms.

❖ Fenretinide functional effect on CFTR is enhanced when cells are co-treated with Zinc, and further enhanced in the presence of VX-809 correction.

❖ These data demonstrate the importance of ceramide microdomains as protective mechanism for the CFTR during cell stress, and suggest a potentiating effect of fenretinide when combined with a CFTR corrector, via a novel mechanism targeting the composition of membrane lipid rafts.