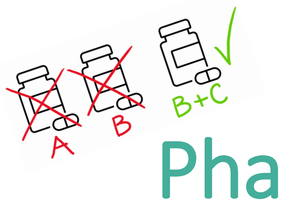
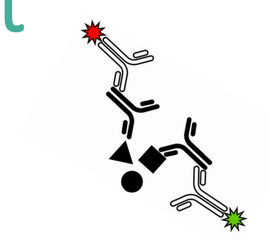


Pharmacological Analysis of a Rare Variant of the Cystic Fibrosis Transmembrane Conductance Regulator via Immunoblot



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1. Abstract

Cystic Fibrosis (CF) can be caused by different mutations, leading to dysfunctional protein variants. Our protein variant of interest is CFTR-E1104X and is caused by a nonsense mutation, which makes the protein shorter. A 2-component treatment tested by Sharma et al. is expected to help cells with the variant of interest produce a full-length protein ("rescue"). In this project, said treatment was tested *in vitro* on E1104X-transfected mammalian cells to assess its potential. The protein variant was rescued, but the treatment needs improvement.

2. Introduction

The Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is a chloride channel mostly present in epithelial cells. On an immunoblot, wild-type CFTR proteins appear separated into different bands in the high molecular weight zone, representing different maturation levels [1]. CF-causing protein variants might lack mature CFTR bands. A mature band appears higher than an immature one [2].

For the most frequent CF-causing CFTR protein variant, CFTR-ΔF508, which is misfolded, a 3-component medication has been successful in recent years [1]. In comparison, for the rare CFTR-E1104X variant ("T1"), which is shortened due to a Premature Termination Codon (PTC) (or nonsense-mutation), there is no approved medication. It is currently gaining attention in North Africa, where it has been found more frequently [3]. A 2-component treatment consisting of the chemicals G418 and SRI-41315 has been tested on other nonsense variants. Its mechanism rescues the variants by synergistically helping the ribosome bypass the PTC during translation (translational readthrough). In this way, a full-length CFTR protein can be synthesised [4].

3. Aim and Leading Questions

Aim: To test whether a combination of G418 with SRI-41315 can rescue E1104X in transfected mammalian cells without natural CFTR expression and to quantify the rescue with n=3 experiments under the same conditions, each with a control group of cells treated with Dimethyl Sulfoxide (DMSO)

Questions: To what extent can the rare nonsense CFTR variant E1104X be pharmacologically rescued, and how do its protein expression and maturation patterns compare to wild-type CFTR ("WT")?

4. Methods

By mammalian cell culture and transfection, the wild-type and variant CFTR Proteins were amplified. One group of cells was treated with the 2-component treatment while the other group was treated with DMSO (= the solvent of the 2-component treatment). Treated cells were lysed to release all of the cell's protein, which was then separated by electrophoresis. The CFTR proteins as well as a common housekeeping protein as loading control (to confirm total protein amount is equal in all lanes) were detected via immunoblot with fluorescence-tagged antibodies which emit light in the IR and NIR spectrum. Intensities of the fluorescent bands were measured with adequate software. The measurement data from the triplicate was used to perform Welch's t-test and generate bar plots.

5. Results

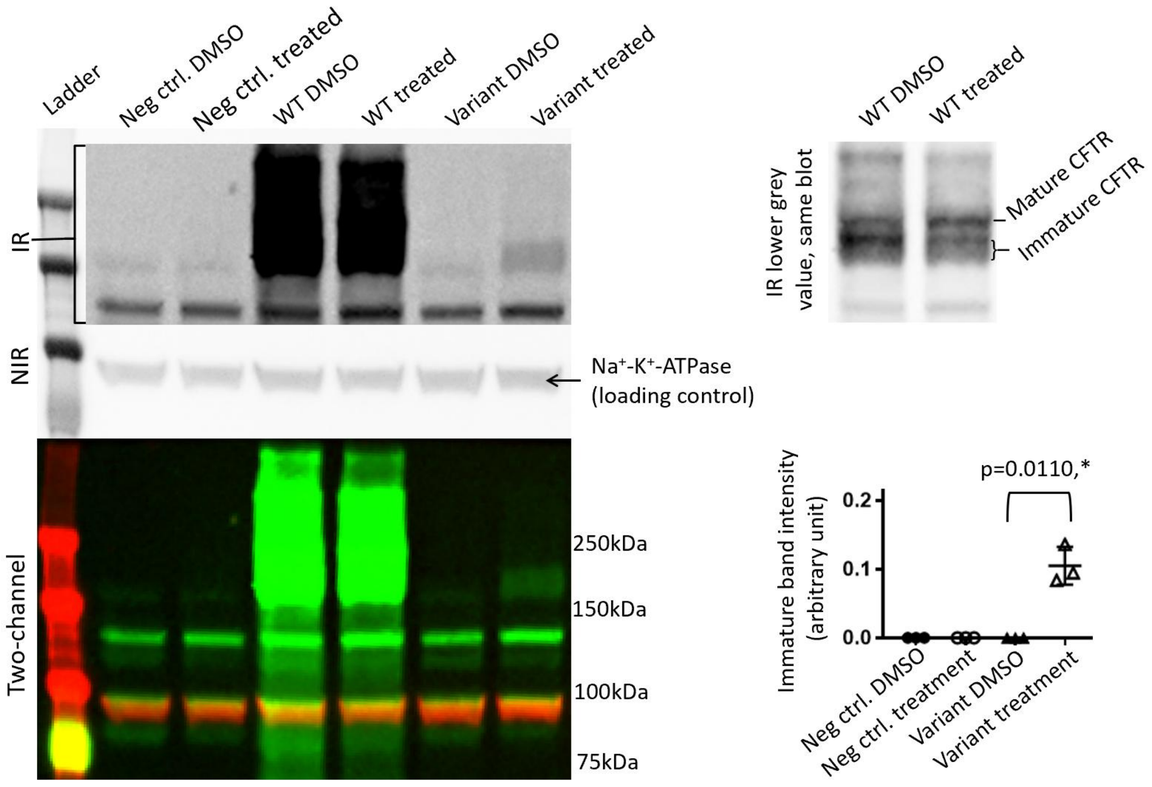

Figure 1: Immunoblot representative of the triplicate, showing CFTR bands in the IR channel (green) and Na^+-K^+-ATPase bands in the NIR channel (red)

6. Discussion

In conclusion, the nonsense variant E1104X was rescued ("T1 treated") to the extent that its immunoblotting signal can be distinguished from background noise (T1 DMSO) on an IR/NIR image, with the p-value of 0.0110 indicating a 1.1% probability that this occurred by chance. The protein expression and maturation pattern of "T1 treated" compared to "WT treated" is reduced regarding IR signal intensity and shows a lack of mature CFTR.

The combination treatment seems to help produce a full-length CFTR, but it still needs to be improved to reduce the difference between the band patterns of the treated variant and the wild-type CFTR. Further tests should then be done on treated cells to see whether the rescued CFTR is present at the cell membrane and whether it in fact allows chloride ion transport.

References

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[2] McClure et al. (2016). Trafficking and function of the cystic fibrosis transmembrane conductance regulator: A complex network of posttranslational modifications. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *311*(4), L719 L733. https://doi.org/10.1152/ajplung.00431.2015

[3] El Makhzen et al. (2024). The burden of cystic fibrosis in North Africa. *Frontiers in Genetics*, *14*, 1295008. https://doi.org/10.3389/fgene.2023.1295008

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Figures

Figure 1: own work