Characterising a panel of rare CF-causing mutations

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Introduction

Cystic Fibrosis (CF) is a debilitating hereditary disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, which codes for an anion channel. > 200 CF-causing mutations have been identified (www.cftr2.org). We have constructed a panel of 69 plasmids carrying rare missense and late nonsense CF-causing mutations, which can be readily deployed in a fluorescence cell-based assay to screen approved and novel CFTR-targeting compounds.

Method

Yellow fluorescent protein (YFP)-CFTR, in which halide-sensitive YFP (1) is tagged to the N-terminal of CFTR (2), was expressed in HEK293 cells. In the presence of high extracellular [I^-], open CFTR channels provide a pathway for I^- influx, which results in YFP quenching (2). The rate of YFP quenching informs on CFTR activity. YFP-CFTR was expressed from a pIRES2-mCherry plasmid. The internal ribosome entry site (IRES) allows transcription of one mRNA encoding both YFP-CFTR and mCherry, but translation of the two as separate proteins. mCherry, expressed in the cytosol, allows accurate positioning of the cell membrane within microscopic images, so the amount of YFP-CFTR at the membrane can be quantified. This assay, therefore, provides information on both the gating and trafficking of CFTR.

Results

A panel of 69 rare CF-mutations has been screened to characterise the type of defect (gating or trafficking), and for quantifying responses to known correctors and potentiators of CFTR.

Conclusions

This screening panel can be used to rapidly profile genotypes to identify those that could benefit from specific CFTR-targeted therapy. Some of these mutations are found in as few as 5 patients in the UK. Clinical trials remain a hurdle, but quantifying responses by different genotypes is a first step towards newly approved CFTR correctors and potentiators reaching the maximal number of patients.

References

(1) Galietta et al. (2001). *Am. J. Physiol. Cell. Physiol.* **281**: C1734-C1742

(2) Langron et al. (2017). *Br. J. Pharmacol.* **174**: 525:539.

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