SiRNA-mediated Knockdown of CFTR in A549 Cells is Associated with Increased Expression of IL-6 and Defective Killing in Response to *Aspergillus fumigatus* Conidia

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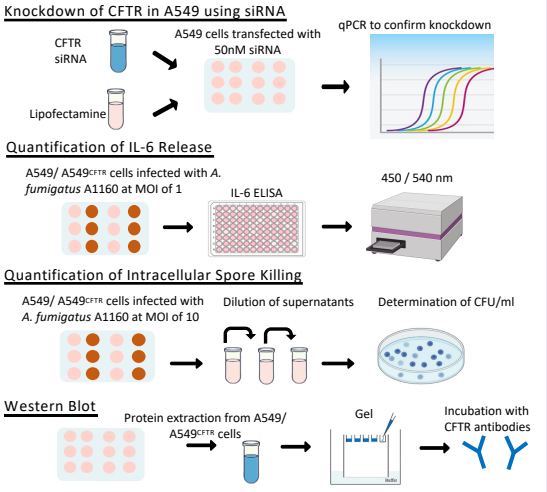
Introduction

Cystic fibrosis (CF) is one of the **most prevalent genetic disorders** found in Caucasian populations[1]. Dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) contributes to the **increased permissibility of the lung to chronic microbial infections**[2]. *Aspergillus fumigatus* is a frequent coloniser of the CF lung causing several diseases including **allergic bronchopulmonary aspergillosis** (ABPA) and **aspergillosis bronchitis** (AB)[3]. We hypothesise that the increased incidence and pathogenicity of *A. fumigatus* infections in CF patients is due to the host's inability to effectively clear fungal conidia as a result of CFTR- mediated cytokine dysregulation and defective killing of spores.

Methods



Results

Confirmation of CFTR Production in A549 Cells

Results from a western blot (Fig 1) confirmed that A549 cells produce CFTR, as indicated by the bands seen at ~165kDa. Calu-3 cells, which are known to produce CFTR were used as a control and produced bands of a similar size. GAPDH was also included as a loading control.

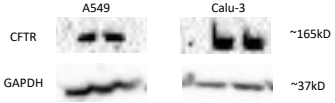


Figure 1. Western blot analysis of CFTR (cystic fibrosis transmembrane conductance regulator) in A549 and Calu-3 cells. Protein was extracted from the cells and CFTR was detected using monoclonal antibodies. Bands at ~165kD indicated the presence of CFTR protein. GAPDH (~37kD) was also included as a loading control.

SiRNA-mediated Knockdown of CFTR in A549 cells is Associated with Increased IL-6 Expression

Analysis of IL-6 production by A549^{CFTR} and A549 cells in response to challenge with *A. fumigatus* conidia was carried out via ELISA. The results revealed that knockdown of CFTR in A549 cells was associated with increased production of IL-6 (Fig 2). For all time points, the release of IL-6 in response to *A. fumigatus* was significantly higher for the A549^{CFTR} cells compared to the wildtype ($P< 0.00001$, $P< 0.05$ and $P<0.05$ for 6, 9 and 16h respectively). Interestingly, there was also a significant difference ($P< 0.001$) in IL-6 production between the A549 and A549^{CFTR} cells in the absence of *A. fumigatus* at 6h (a mean IL-6 production of 3.8 and 5.2 pg/ml respectively.

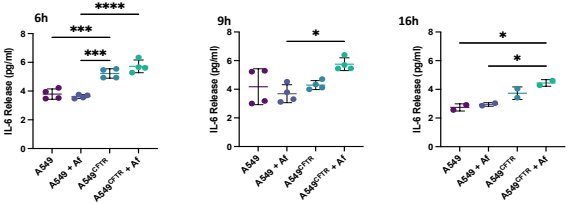


Figure 2. IL-6 cytokine release of A549 and A549^{CFTR} (siRNA) cells following infection with *A. fumigatus*. Cells were infected with A1160 *A. fumigatus* conidia and supernatants were collected after 6h, 9h and 16h and assayed by ELISA. Data in graphs are represented as mean ± standard deviation. Means were compared; *$P < 0.05$. **$P < 0.001$, ***$P < 0.0001$ and **** $P< 0.00001$.

Results

SiRNA-mediated knockdown of CFTR is Associated with Defective Intracellular Killing

A549 and A549^{CFTR} cells were infected with *A. fumigatus* conidia and the percentage that were intracellularly killed was quantified. Overall, a higher number of viable spores were recovered from the A549^{CFTR} cells compared to the A549 controls. At 6h, the number of viable spores recovered was 1.6x higher for the A549^{CFTR} cells, while at 9h, a 1.4x fold increase was observed.

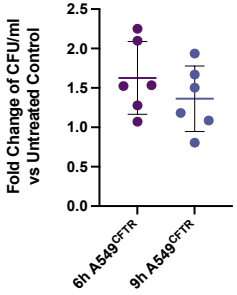


Figure 3. The percentage of viable *A. fumigatus* spores following incubation with A549 and A549^{CFTR} (siRNA) for 6 and 9h is represented as fold change vs. control (A549 cells infected with *A. fumigatus*). Data in graphs are represented as mean ± standard deviation.

Conclusion

- **SiRNA-mediated knockdown cell lines can be suitable as *in vitro* models** for investigating host-fungal pathogen interactions in the CF lung.
- **SiRNA-mediated CFTR knockdow**n in A549 cells is associated with **increased IL-6 production** in response to *A. fumigatus* conidia.
- **SiRNA-mediated CFTR knockdown** is associated with **increased viability of *A. fumigatus* conidia**

References and Acknowledgements

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