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Short Communication

Expression of the inflammatory regulator A20 correlates with lung function in patients with cystic fibrosis

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Abstract

Background: A20 and TAX1BP1 interact to negatively regulate NF-κB-driven inflammation. A20 expression is altered in F508del/F508del patients. Here we explore the effect of CFTR and CFTR genotype on A20 and TAX1BP1 expression. The relationship with lung function is also assessed.
Methods: Primary nasal epithelial cells (NECs) from CF patients (F508del/F508del, $n=7$, R117H/F508del, $n=6$) and controls (age-matched, $n=8$), and 16HBE14o- cells were investigated. A20 and TAX1BP1 gene expression was determined by qPCR.
Results: Silencing of CFTR reduced basal A20 expression. Following LPS stimulation A20 and TAX1BP1 expression was induced in control NECs and reduced in CF NECs, broadly reflecting the CF genotype: F508del/F508del had lower expression than R117H/F508del. A20, but not TAX1BP1 expression, was proportional to FEV_1 in all CF patients ($r=0.968$, $p<0.001$).
Conclusions: A20 expression is reduced in CF and is proportional to FEV_1. Pending confirmation in a larger study, A20 may prove a novel predictor of CF inflammation/disease severity.

Keywords: A20 protein; FEV_1; NF kappa B; Airway epithelial cells; Cystic Fibrosis; Chronic inflammation

1. Introduction

Cystic Fibrosis (CF) lung disease is heterogenous in nature and the severity of disease in patients with the same genotype can vary significantly. Activation of TLR4 by Gram-negative bacteria such as *Pseudomonas aeruginosa* (*P. aeruginosa*) leads to pro-longed NF-κB (p65) signalling in CF primary epithelial cells and CF cell lines [1,2]. A20 (*TNFAIP3*, tumor necrosis factor, alpha-induced protein 3) is an endogenous negative regulator of the NF-κB pathway and is rapidly and transiently induced in response to bacterial and viral stimuli. A20 terminates NF-κB driven inflammation by inhibiting the polyubiquitination and activation of the central adaptor protein TNF receptor-associated factor (TRAF) 6 [3]. Desensitized pathogen recognition is thought to be secondary to hyperactive TRAF6 signalling in chronic lung diseases including CF [4].

Despite varied pathogeneses, A20 is a critical regulator of inflammatory processes in rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes, multiple sclerosis, psoriasis and inflammatory bowel disease [5]. Furthermore, A20 is a susceptibility gene for the development of Crohn's [6] and irritable bowel disease [7]. In the lung, *P. aeruginosa* challenge rapidly induces A20 in mice [8], while A20 is essential for termination of TLR2/4 mediated IL-8 release from primary airway epithelial cells [9]. We recently showed that A20 is reduced in CF airway epithelial cells after stimulation with LPS. The action of A20 is dependent on an adapter protein called TAX1BP1, which is also significantly reduced in LPS-treated CF cells [1,10]. Furthermore, this work suggested that CF airway epithelial cells lack

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essential interactions between A20, TAX1BP1 and TRAF6, which may partly explain the associated chronic activation of NF-κB [1].

Here we further investigate the relationship between A20, TAX1BP1 and CFTR as well as any association with lung function (FEV_1) in patients homozygous for F508del and heterozygous for R117H/F508del.

2. Materials and methods

Primary nasal epithelial cells (NEC) were obtained from CF patients (F508del/F508del {$n=7$}, R117H/F508del {$n=6$}) and age matched controls {$n=8$} as previously described [11] and were fully differentiated at air–liquid interface (ALI). NECs were treated with LPS (*P. aeruginosa*, Sigma, 50 μg/ml) for 24 h. The Research Ethics Committee of Northern Ireland approved the study and all participants provided informed consent (07/NIR02/23). In CF patients lung function (FEV_1) was also determined at time of cell sampling.

The 16HBE14o- bronchial epithelial cell line (D. Gruenert UCSF, USA) was grown in submersion and transfected at 80% confluence with commercially available CFTR siRNA (GenomeWide siRNA, Qiagen) and RNAiFect™ Transfection Reagent (Qiagen). Experiments included mock (transfection reagent only) and scrambled (Allstars Neg siRNA, Qiagen) controls. Transfection efficiency was confirmed by qPCR as 72%±5.34%.

Total RNA was extracted using an RNeasy kit (Qiagen) and quantified on a Nanodrop (Thermo Scientific). Equal amounts of RNA were reverse transcribed into cDNA (Sensiscript Reverse

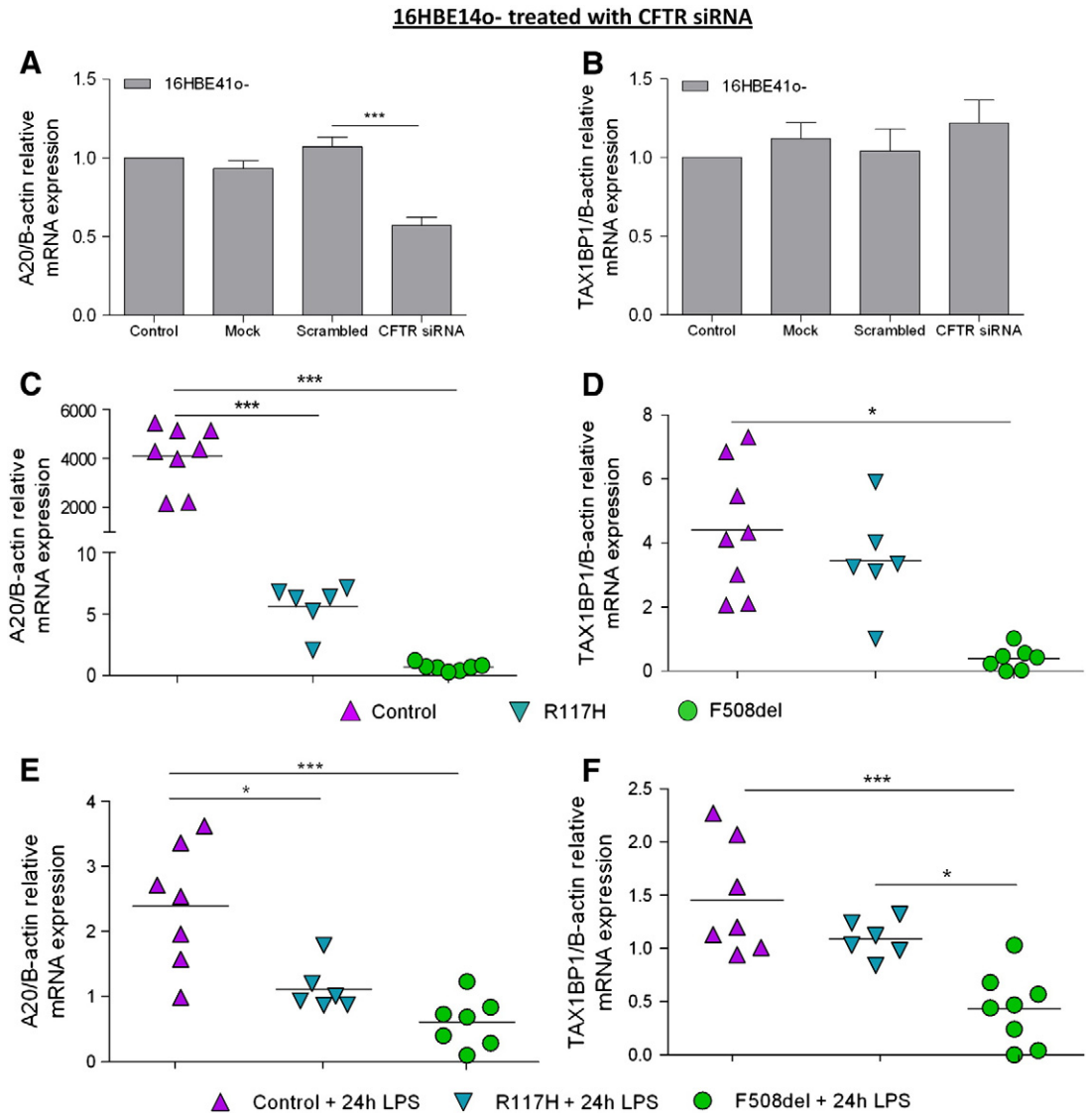

Fig. 1. The effect of CFTR and genotype on A20 and TAX1BP1 mRNA expression. The basal expression of A20 (A) and TAX1BP1 (B) was assessed by qPCR in 16HBE14o- cells treated with siRNA against CFTR. Data are presented as mean±SEM with $n=3$. ***$p<0.001$ compared with scrambled siRNA. The effect of different CF genotypes (R117H/F508del and F508del/F508del) on basal A20 (C) and TAX1BP1 (D) mRNA expression was determined by qPCR. Basal expression was expressed relative to Jurkat cells, which acted as an internal calibrator for all experiments. Furthermore, the mRNA expression of LPS-induced A20 (E) and TAX1BP1 (F) was also determined by qPCR. Expression was calculated after 24 h simulation with LPS and data are presented as mean±SEM with $n=6$–8 as indicated in the figure. *$p<0.05$, ***$p<0.001$ between groups as indicated in the figure.