ELSEVIER
Journal of Cystic Fibrosis xx (2018) xxx – xxx
Journal of Cystic Fibrosis
www.elsevier.com/locate/jcf

Original Article

Cystic fibrosis epithelial cells are primed for apoptosis as a result of increased Fas (CD95)

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Received 7 July 2017; revised 11 January 2018; accepted 30 January 2018
Available online xxxx

Abstract

Background: Previous work suggests that apoptosis is dysfunctional in cystic fibrosis (CF) airways with conflicting results. We evaluated the relationship between dysfunctional cystic fibrosis transmembrane conductance regulator (CFTR) and apoptosis in CF airway epithelial cells.
Methods: Apoptosis and associated caspase activity were analysed in non-CF and CF tracheal and bronchial epithelial cell lines.
Results: Basal levels of apoptosis and activity of caspase-3 and caspase-8 were significantly increased in CF epithelial cells compared to controls, suggesting involvement of extrinsic apoptosis signalling, which is mediated by the activation of death receptors, such as Fas (CD95). Increased levels of Fas were observed in CF epithelial cells and bronchial brushings from CF patients compared to non-CF controls. Neutralisation of Fas significantly inhibited caspase-3 activity in CF epithelial cells compared to untreated cells. In addition, activation of Fas significantly increased caspase-3 activity and apoptosis in CF epithelial cells compared to control cells.
Conclusions: Overall, these results suggest that CF airway epithelial cells are more sensitive to apoptosis via increased levels of Fas and subsequent activation of the Fas death receptor pathway, which may be associated with dysfunctional CFTR.

Keywords: Epithelial cells; Apoptosis; Fas

1. Introduction

Cystic fibrosis (CF) is an autosomal recessive genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. It affects all exocrine organs including the airways, pancreas, intestine and sweat glands, however, pulmonary disease causes most of the morbidity and mortality associated with CF [1]. CFTR – a 1480 amino acid protein that functions as a chloride channel – is highly expressed in the airway epithelium where it regulates chloride ion movement [2]. A single deletion of a phenylalanine residue at position 508 in CFTR (ΔF508-CFTR) results in a misfolded protein that is associated with approximately 90% of CF cases [3]. CF lung disease is associated with the failure of pulmonary host defence leading to a vicious cycle of continual infection, inflammation and remodelling of lung tissue. The airway epithelium plays a key role in pulmonary host defence, and apoptosis is a physiological process essential for homeostasis of epithelial function.

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https://doi.org/10.1016/j.jcf.2018.01.010
1569-1993/

Apoptosis, or programmed cell death, is a form of regulated cell death in which activation of specific proteases called caspases leads to DNA cleavage and cell death. Previous work suggests that apoptosis is dysfunctional in the CF airways with conflicting results. While some reports describe defective apoptosis of epithelial cells expressing mutant forms of CFTR [4–6], a number of others report excessive apoptosis in CF cells [7–13]. In addition, it is unclear how CFTR misfolding and dysfunction contributes to apoptosis or the susceptibility of cells to pro-apoptotic stimuli. Nonetheless, accumulation of apoptotic cells as evident in the CF lung may precipitate chronic inflammation and progressive airway damage [14,15].

Previous studies have focused on the effect of external stimuli on apoptosis in CF epithelium [8,16]. However, we now show that in basal (unstimulated) CF epithelial cells, indices of apoptosis are increased (caspase-3, caspase-8). Further evaluation of upstream apoptotic factors demonstrated increased expression of Fas which contributed to the increased activation of caspase-3 and -8 demonstrated in these cells. Treatment of CF cells with Fas activating antibody, CH-11, resulted in a significant increase in caspase-3 and -8 activation as well as significant increase in apoptosis in these cells compared to non-CF cells. Increased Fas expression was related to CFTR function as treatment of primary non-CF bronchial epithelial cells with a CFTR inhibitor resulted in increased Fas expression in these cells. These findings indicate that CF epithelium is in a primed condition for apoptosis which results in significant programmed cell death upon activation. These finding may impact on effective innate host defence function offered by the epithelial barrier in the CF lung.

2. Methods and materials

2.1. Cell culture

The human bronchial epithelial cell line (16HBE14o-, HBE), CF bronchial epithelial cell line (CFBE41o-, CFBE) homozygous for the ΔF508 mutation, the human tracheal epithelial cell line (9HTEo-, HTE) and the CF tracheal epithelial cell line (CFTE29o-, CFTE) homozygous for the ΔF508 mutation were obtained as a gift from Prof Dieter Gruenert (California Pacific Medical Center Research Institute, San Francisco, CA, USA). Cell lines were maintained in Minimum Essential Medium supplemented with 10% heat-inactivated foetal calf serum, 2 mM L-glutamine and 1% penicillin/streptomycin (PAA Laboratories, Somerset, UK) at 37 °C under 5% CO_2. Normal human bronchial/tracheal epithelial cells (NHBE) were obtained from Lonza (Basel, Switzerland) and were cultured according to provided guidelines.

For experiments, non-CF (HBE, HTE) and CF epithelial cell lines (CFBE, CFTE) were adhered overnight. Cell-free supernatants were collected following a further 24 h in fresh media. Whole cell lysates for protease activity assays and Western blotting were prepared as described below. Total RNA was isolated from cells using TRI Reagent® (Sigma-Aldrich) as per manufacturer's instructions.

2.2. Cell treatments

For caspase-8 inhibitor experiments, CF epithelial cells (CFTE, CFBE) were left untreated or treated with Ac-IETD-CHO (10 μM, Enzo Life Sciences, Inc., Exeter, UK) for 24 h. For CFTR inhibitor experiments, non-CF epithelial cells (HTE, HBE, NHBE) were treated ± CFTR inhibitor ($CFTR_{inh\text{-}172}$, 10–20 μM) for 24 h. For Fas activation experiments, non-CF and CF epithelial cells were treated ± a human Fas activating antibody (clone CH-11, 0.5 μg/ml, Millipore, Darmstadt, Germany) for 24 h. For Fas neutralisation experiments, cells were treated with 100 ng/ml anti-Fas neutralising antibody (clone ZB4, Millipore) or corresponding isotype control mouse (IgG_1, R&D Systems Europe Ltd., Abingdon, UK) for 6 h.

2.3. Bronchial brushing analysis and study approval

Following informed consent under a protocol approved by Beaumont Hospital Institutional Review Board, bronchial brushings were sampled from in individuals with CF (n = 3) and non-CF controls (n = 3). Total RNA was isolated and reverse transcribed as previously described [17].

2.4. Apoptosis detection

Non-CF and CF epithelial cells were treated ± human Fas activating antibody (clone CH-11, 0.5 μg/ml, Merck Millipore, Darmstadt, Germany) for 24 h. Cellular apoptosis (DNA fragmentation) was quantified using the TiterTACS™ Colorimetric Apoptosis Detection Kit (Trevigen, Gaithersburg, MD). Samples were fixed and labelled as per manufacturer's instructions. The $Abs_{630\ nm}$ of the wells was measured in a 96-well microplate reader (Synergy HT using Gen5™ software, BioTek UK).

2.5. Preparation of whole cell lysates - caspase activity assays and Western blotting

Cells were lysed on ice in lysis buffer (50 mM Tris-HCL pH 7.5, 150 mM NaCl, 5 mM EDTA, 0.2% Igepal) for caspase activity assays or in RIPA buffer (50 mm Tris-HCl, pH 7.5, 150 mm NaCl, 1% Nonidet P-40, 0.25% sodium deoxycholate, 1 mm EDTA, 1% Triton X-100) supplemented with protease and phosphatase inhibitor cocktails (Roche Applied Science, UK) for Western blotting [18]. Cell lysates were centrifuged at 13,000 ×*g* for 10 min to remove insoluble debris and stored at −80 °C until required. Total protein concentrations were determined using the BCA method (Pierce BCA Assay, Fisher Scientific, Leicestershire, UK). Cellular caspase activity was determined using a panel of fluorogenic substrates: Ac-Asp-Glu-Val-Asp-7-Amino-4-methylcoumarin (Ac-DEVD-AMC) for caspase-3 and Ac-Ile-Glu-Thr-Asp-AMC (Ac-IETD-AMC) for caspase-8 (Enzo Life Sciences, Inc., Exeter, UK). Samples were incubated with substrate (50 μM) and fluorescence (substrate turnover) was determined by excitation at 360 nm and emission at 460 nm in a 96-well microplate reader (Synergy HT using Gen5™ software, BioTek UK). The rate of

Please cite this article as: Chen Q, et al, Cystic fibrosis epithelial cells are primed for apoptosis as a result of increased Fas (CD95), J Cyst Fibros (2018), https://doi.org/10.1016/j.jcf.2018.01.010