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A novel regulatory role for tissue transglutaminase in epithelial-mesenchymal transition in cystic fibrosis

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ABSTRACT

Cystic fibrosis (CF) is a genetic disorder caused by mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) for which there is no overall effective treatment. Recent work indicates tissue transglutaminase (TG2) plays a pivotal intracellular role in proteostasis in CF epithelia and that the pan TG inhibitor cysteamine improves CFTR stability. Here we show TG2 has another role in CF pathology linked with TGFβ1 activation and signalling, induction of epithelial-mesenchymal transition (EMT), CFTR stability and induction of matrix deposition. We show that increased TG2 expression in normal and CF bronchial epithelial cells increases TGFβ1 levels, promoting EMT progression, and impairs tight junctions as measured by Transepithelial Electric Resistance (TEER) which can be reversed by selective inhibition of TG2 with an observed increase in CFTR stability. Our data indicate that selective inhibition of TG2 provides a potential therapeutic avenue for reducing fibrosis and increasing CFTR stability in CF.

1. Introduction

Cystic fibrosis (CF) is a genetic disorder caused by mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) [1]. Of the 1900 mutations now recognised to be present in CFTR the most common is F508del, all of which lead to the abnormal transport of chloride and sodium across the epithelium, resulting in multiple clinical manifestations of which lung disease characterised by chronic lung obstruction, infection and inflammation is the major cause of morbidity. CF affects not only the lungs, but also the pancreas, liver and intestines. True to its terminology fibrosis of the pancreas was noted when the disease was first recognised and uncontrolled airway remodelling is now recognised to start early in life and is linked to a poor pulmonary outcome with extensive fibrosis [2]. Transforming growth factor β1 (TGFβ1) is a key growth factors involved in fibrosis, including cystic fibrosis where increased levels are reported in lungs [3]. In addition to its pro-inflammatory properties TGFβ1 has been shown recently to inhibit the biosynthesis of CFTR and also prevents the biofunctional rescue of CFTR, especially the F508del [4]. Importantly over-expression of TGFβ1 in the CF lung has been associated with a more severe CF phenotype.

Current treatment for CF is limited and largely confined to the relief of patient symptoms. This includes use of anti-inflammatory agents and antibiotics to suppress microbial infection. Lung transplantation may be necessary as the condition progresses, but both lungs need to be, replaced to prevent infection. Recently, new therapeutic avenues have been applied to CF treatment, such as the use of CFTR correctors and potentiators to improve the cell processing and function of the mutant CFTR [5]. For example some small compounds can correct the function of F508del-CFTR in CF cells by improving its folding in the ER and stabilizing the protein during its secretion to the cell surface. Importantly in cell models, the effect of the compounds can be detected within 3 h. However, one recent corrector that reached a clinical trial, VX-809, failed to rescue the functional defect of F508del-CFTR in nasal epithelium and did not improve lung function in patients. Recently, another family of compounds named CFTR potentiators, especially VX-770, have been shown to improve CFTR function in cell models [6]. By increasing the open time of CFTR, VX-770 reduces Na^+ and fluid absorption and therefore prevents the dehydration of the apical surface.

In other studies the application of the pan inhibitor cysteamine has been used in cell and animal models and more recently in early phase clinical trials. When used together with epigallocatechin gallate (EGCG, a green tea flavonoid that inhibits the enzyme Casein Kinase 2), improved and sustained mutant CFTR function was reported in patient nasal epithelial cells and in sweat ducts [7]. However, cysteamine, in addition to its action as a pan but relatively poor inhibitor of transglutaminases, is also an inhibitor of cysteine proteases and has been reported to have a diverse range of other potential targets. Its ability to inhibit cysteine proteases may also be important in preventing CFTR degradation in CF and in the prevention of apoptosis but these actions remain to be proven.

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Nomenclature

CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
TGFβ1	transforming growth factor β1
ECM	extracellular matrix
EGCG	epigallocatechin gallate
TG2	tissue transglutaminase
EMT	epithelial-mesenchymal transition
ALI	air liquid interface
HBEC	primary human bronchial epithelial cells
LTBP1	Latent TGFβ1 binding protein 1
EMT	epithelial-mesenchymal transition
TEER	Trans-Epithelial Electric Resistance
TRE	TGFβ1 Response Element
αSMA	αSmooth Muscle Actin
HBE	human bronchial epithelial
PPARγ	peroxisome proliferator-activated receptor γ
NF-κB	nuclear factor-κB
TNFα	tumour necrosis factor α
FN	fibronectin
rhTGFβ1	recombinant human TGFβ1

The other target of cysteamine – tissue transglutaminase (TG2) is reported to play a pivotal role in proteostasis in CF epithelia demonstrating an intracellular involvement of this multifunctional enzyme in CF [7]. This work proposed that in CF the dysfunctional CFTR induces a reactive oxygen species response which in turn triggers activation of intracellular TG2 leading to protein crosslinking, culminating in blocked autophagy and protein aggregate accumulation. The work also indicated that in the CF inflammatory response, intracellular crosslinking by TG2 inhibited the anti-inflammatory peroxisome proliferator-activated receptor γ (PPARγ), leading to sustained activation of the inflammatory process. Importantly use of the pan transglutaminase inhibitor cysteamine can reverse these observations [7].

In addition to this intracellular role TG2 is also reported to have a number of extracellular functions which generally manifest themselves in a variety of human diseases. Increases in TG2 expression are normally associated with injury or stress resulting in its secretion onto the cell surface and into the extracellular matrix (ECM) through a non-conventional mechanism involving cell surface heparan sulphates [8]. In the ECM Ca^{2+} levels are high resulting in displacement of intracellular regulator GTP, leading to the activation of TG2 where it can contribute to other important CF pathologies. For example, as referred to earlier, during childhood, CF lungs and other tissues like the pancreas, intestine and liver develop fibrosis resulting in the remodelling and dysfunction of these organs as the disease progresses for which there is presently no therapy. Extracellular TG2 is known to be a key player in the development of tissue fibrosis via its ability to crosslink matrix proteins leading to their increased deposition and stability and via its ability to activate matrix bound TGFβ1 [9]. Recent work also suggests that TG2 mediated crosslinking of TGFβ into the matrix may prolong the activity of TGFβ [10]. Moreover, expression of TG2 can be upregulated by TGFβ1 thus, propagating the fibrotic effects of TG2 and increased levels of TGFβ1 are found in the CF lung [11].

TG2 is also reported to be involved in epithelial-mesenchymal transition (EMT) [12]. A process thought to be important in a number of fibrotic conditions [13]. Over-expressed in highly invasive cancers, TG2 mediates EMT via its ability to enhance the activation of NF-κB, TGFβ1 and TNFα [12].

In this paper we have explored the role of TG2 in CF pathology in particular its link with TGFβ1 activation and signalling, induction of EMT, CFTR stability and the induction of fibrosis. We show that the presence of TG2 in both normal and CF bronchial epithelial cells promotes EMT progression leading to increased motility and increased fibronectin (FN) expression and deposition, via a TGFβ1-stimulated pathway involving TG2 mediated matrix bound TGFβ1. We will also show that selective inhibition of TG2 reduces TGFβ1 levels and leads to reversal of this process which is accompanied by an increase in the stability and improvement in CFTR processing in CF airway bronchial epithelial cells.

2. Material and methods

2.1. Cell culture

IB3-1 cells (a mutant tracheal epithelial cell line with a heterozygote mutation, F508del/W1282X, leading to the loss of CFTR channel function and immortalized by SV-40 transfection) and C38 cells ("add back" IB3-1 cell line which has a truncated CFTR channel with 119 residues missing but functional with elevated basal Cl-ion efflux) were obtained from Johns Hopkins University (Baltimore, USA). HBEC (primary human bronchial epithelial cell, isolated from surface epithelium of human bronchi) was a kind gift from Dr. Lindsay Marshall (Aston University, UK). Cells were cultivated in Airway Epithelial cell Medium (AEM) containing bovine pituitary extract, epidermal growth factor, insulin, hydrocortisone, epinephrine, triiodo-L thyronine, transferrin and retinoic acid (Promocell, UK).

2.2. ALI culture

Transwell® inserts with a surface area of 0.33 cm^2 and 0.4 μm pore size were inserted into Transwell® companion plates. 100 μg/ml solution of Collagen IV in 3% (v/v) acetic acid was added to each insert membrane and allowed to attach for 45 min at room temperature. Media was then used to rinse inserts to neutralize the acidity of the solution. 3×10^4 cells/300 μl of AEM media was added to each insert and 600 μl of complete media added to the well underneath each insert. The cells were cultured for 4 days. On the 3rd day, TG2 inhibitors or the TGFβ receptor inhibitor was added and cells incubated for a further 12 h, after which media was removed and the cells then exposed to air. Media in companion Transwell® plates was replaced and the inhibitor introduced. Cells were then cultured for 14 days during which basal medium with or without inhibitor was changed on alternate days. When cells cultured in ALI condition were transfected with TG2 shRNA or TG2 virus all transfections/transductions were undertaken prior to ALI culture.

2.3. Treatment of cell with inhibitors

All TG2 inhibitors used in this study were synthesized in the Aston laboratories. R283 is a specific TG inhibitor not selective for TG2 but shown to penetrate cells [14]. R292, R294 and R281 are peptidic water soluble inhibitors that are cell impermeable [15] and able to differentiate between TG2 and Factor XIIIa. 1-155, 1-33, 2-18 and 1-159 are peptidomimetic inhibitors that are highly selective for TG2 [16,17]. 1-155 is cell permeable and 1-159 is cell impermeable [17]. R283 is used at 500 μM, 1-155 is used at 1 μM or 5 μM, 1-33 is used at 50 μM, 1-159 at 5 or 10 μM, R294 is used at 500 μM, 2-18 is used at 50 μM, R281 and R292 is used at 250 μM. Cells were treated for 48 h with an inhibitor unless otherwise stated. At the time this work was undertaken, the inactivating antibody against TG2 that we have used previously [14] was not readily available due to its pharmaceutical development.

For inhibition of TGFβ signalling the TGFβ receptor inhibitor SB 431542 (Sigma-Aldrich, UK) was used at concentration of 10 μM, which by the XTT assay was shown to be non-toxic to the cells.

2.4. Inhibition of TG2 with TG2 shRNA

5×10^5 cells were seeded into 6-well plates in 2 ml of medium. The cells were incubated overnight. 10 nM of TG2 shRNA (CACAAGGGCGAA