Biochimica et Biophysica Acta 1832 (2013) 2340–2351

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Anoctamin 1 dysregulation alters bronchial epithelial repair in cystic fibrosis

Manon Ruffin [a,b], Mélanie Voland [a,b], Solenne Marie [a,b], Monique Bonora [a,b], Elise Blanchard [a,b], Sabine Blouquit-Laye [c,d], Emmanuel Naline [c,d], Philippe Puyo [e], Philippe Le Rouzic [a,b], Loic Guillot [a,b], Harriet Corvol [a,b,f], Annick Clement [a,b,f], Olivier Tabary [a,b,*]

[a] Inserm, U938, 34 Rue Crozatier, 75012 Paris, France
[b] UPMC, University of Paris 06, 4 Place Jussieu, 75005 Paris, France
[c] Laboratoire de Pharmacologie, UPRES EA220, 11 Rue Guillaume Lenoir, 92150 Suresnes, France
[d] Université Versailles Saint Quentin en Yvelines, UFR Sciences de la santé, Montigny-le-Bretonneux, France
[e] Hôpital Foch, Service de Chirurgie Thoracique et Transplantation Pulmonaire, Suresnes, France
[f] APHP, Hôpital Trousseau, Pediatric Pulmonary Department, 26 Avenue du Docteur Arnold-Netter, 75012 Paris, France

ARTICLE INFO

Article history:
Received 6 May 2013
Received in revised form 30 August 2013
Accepted 19 September 2013
Available online 27 September 2013

Keywords:
Anoctamin 1
TMEM16a
Cystic fibrosis
Airway
Cell proliferation
Bronchial epithelial repair

ABSTRACT

Cystic fibrosis (CF) airway epithelium is constantly subjected to injury events due to chronic infection and inflammation. Moreover, abnormalities in CF airway epithelium repair have been described and contribute to the lung function decline seen in CF patients. In the last past years, it has been proposed that anoctamin 1 (ANO1), a Ca^{2+}-activated Cl^- channel, might offset the CFTR deficiency but this protein has not been characterized in CF airways. Interestingly, recent evidence indicates a role for ANO1 in cell proliferation and tumor growth. Our aims were to study non-CF and CF bronchial epithelial repair and to determine whether ANO1 is involved in airway epithelial repair. Here, we showed, with human bronchial epithelial cell lines and primary cells, that both cell proliferation and migration during epithelial repair are delayed in CF compared to non-CF cells. We then demonstrated that ANO1 Cl^- channel activity was significantly decreased in CF versus non-CF cells. To explain this decreased Cl^- channel activity in CF context, we compared ANO1 expression in non-CF vs. CF bronchial epithelial cell lines and primary cells, in lung explants from wild-type vs. F508del mice and non-CF vs. CF patients. In all these models, ANO1 expression was markedly lower in CF compared to non-CF. Finally, we established that ANO1 inhibition or overexpression was associated respectively with decreases and increases in cell proliferation and migration. In summary, our study demonstrates involvement of ANO1 decreased activity and expression in abnormal CF airway epithelial repair and suggests that ANO1 correction may improve this process.

1. Introduction

Cystic fibrosis (CF) is an autosomal recessive disease that is due to mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene encoding an essential cAMP-dependent chloride (Cl^-) channel. Defective CFTR function in the airway epithelium leads to lung alterations, the main cause of morbidity and mortality of CF patients.

In CF airway epithelial cells, CFTR impairment results in decreased Cl^- secretion and increased sodium (Na^+) influx, leading to an increase in water absorption that translates into greater viscidity of the airway surface liquid. In advanced CF, defective ciliary clearance and mucus hypersecretion cause airway obstruction and produce a favorable environment to chronic infection and inflammation, resulting in epithelial injury and lung function impairments [1]. In addition, abnormalities in epithelial injury repair in CF airways have been described [2] and induce characteristic epithelial remodeling [3]. Several studies have established involvement of interleukin-8, some metalloproteinases, and CFTR Cl^- activity in the epithelial repair process, but the underlying mechanisms remain unclear [2,4,5].

Chloride ion movements across the cell membrane are tightly regulated by Cl^- channels and transporters, which remain poorly characterized in the lungs, the only exception being the CFTR channel. CFTR is the main gateway for Cl^- secretion in the human airway epithelium. However, alternative Cl^- conductance associated with channels called calcium-activated chloride channels (CaCCs) has been identified in several cell types, including those of secretory epithelia [6,7]. Different authors have compared CaCC activities in human nasal cells and have observed an increased in CF compared to non-CF group [8–11]. For

Abbreviations: ANO1, anoctamin 1; CaCC, calcium-activated chloride channel; CF, cystic fibrosis; CI, normalized cell index; F508del, phenylalanine 508 deletion; RTCA, real time cell analysis; TMEM16a, transmembrane 16a protein; WGA, wheat germ agglutinin

* Corresponding author at: Hôpital St. Antoine, Inserm U938, Bât. Kourilsky 5ème Étage, 34 Rue Crozatier, 75012 Paris, France. Tel.: +33 1 49 28 46 23.
E-mail address: olivier.tabary@inserm.fr (O. Tabary).

0925-4439/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbadis.2013.09.012

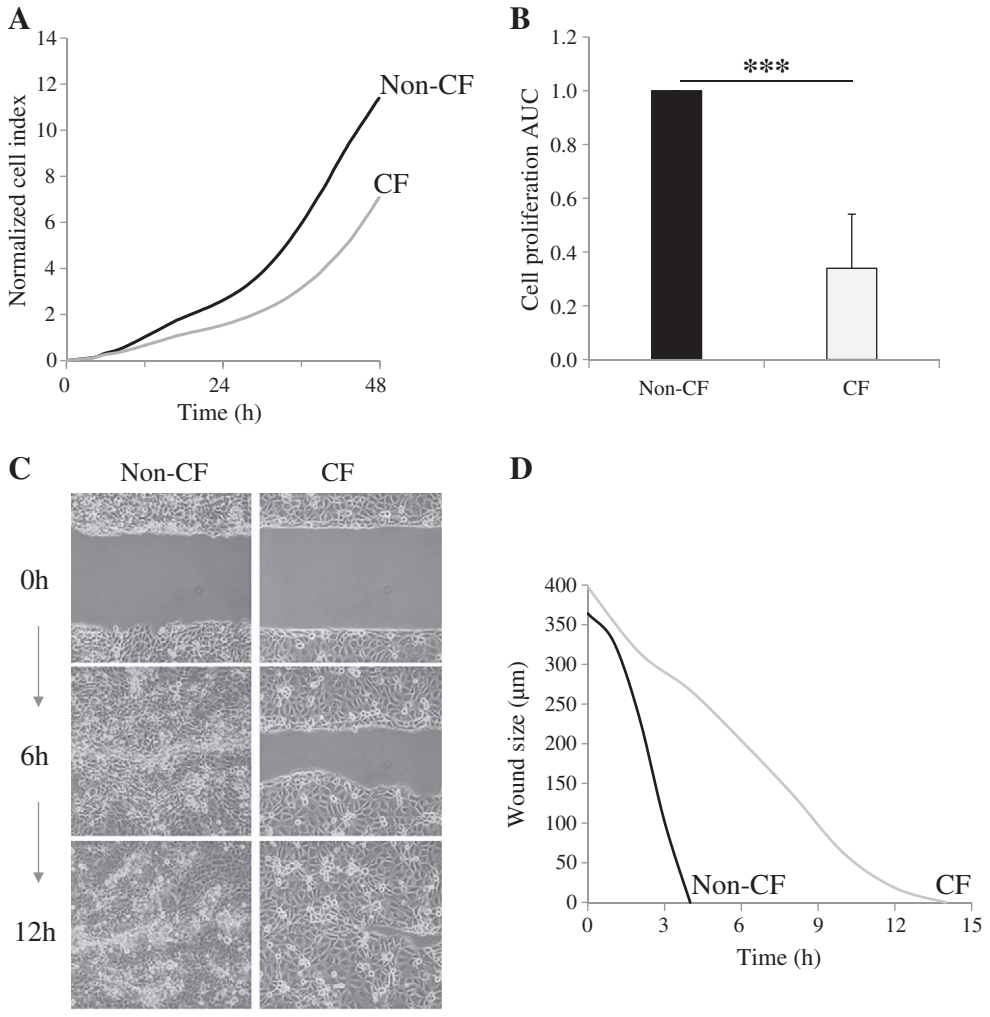

Fig. 1. Cell proliferation and migration of non-CF and CF bronchial epithelial cell lines in basal conditions. A) Original traces of non-CF and CF cell line proliferation monitored for 48 h under basal conditions using real time cell analysis ($n = 8, p < 0.001$). B) Quantification of cell proliferation within 48 h performed with area-under-the-curve (AUC) values normalized to non-CF cells. C) Representative photographs taken every 6 h during 12 h of wound closure of non-CF and CF cell lines under basal conditions. D) Wound closure kinetics of non-CF and CF cell lines under basal conditions showed in C.

this reason it has been proposed that CaCCs might compensate for CFTR function impairment in CF patients [11] but the molecular identity of CaCCs remained unknown for many years. CaCCs are activated by purinergic signaling through the binding of ATP/UTP to $P2Y_2$ receptor that induces an increase of intracellular Ca^{2+} concentration. Based on this mechanism, a $P2Y_2$ agonist (denufosol tetrasodium) has been developed and tested in a clinical trial for CF patients. Despite the positive effects on lung function that have been observed in phase II of the clinical trial, phase III failed to show a significant difference in lung function of patients treated with denufosol or placebo [12,13]. This study outcome might be due to the short half-life and duration of action of the molecule [14]. Design of specific CaCC activators could improve stimulation; therefore CaCC molecular identification was necessary.

In 2008, three research teams identified one potential CaCC candidate called ANO1 (anoctamin 1) or TMEM16a (transmembrane protein 16a) [15–17]. This protein exhibits all the functional characteristics of CaCCs, especially regarding the anion permeability sequence and Ca^{2+} dependence. ANO1 is a transmembrane protein expressed in secretory epithelia including the airway surface epithelium [18–20]. Mice lacking ANO1 exhibit decreased Ca^{2+}-activated Cl^- secretion and impaired mucociliary clearance, indicating a substantial physiological role for ANO1 in the airways [21,22]. Moreover, ANO1 is involved in mucin production [23], HCO_3^- permeability [24] and cytokine secretion [25]. Taken together, these results indicate that ANO1 is a CaCC and may be capable to restore Cl^- efflux and other important functions dysregulated in CF airways and could be in a relationship with CFTR protein [26].

Furthermore, ANO1, which is located on the amplified region 11q13, is overexpressed in many cancer cells [27–29]. Thus, two different groups showed that ANO1 inhibition decreased the proliferation of a human pancreatic cancer cell line [30] and suppressed the growth and invasiveness of human prostate cancer cells [31]. Moreover, a recent study showed that ANO1 is a marker of poor prognosis in head and neck squamous cell carcinoma and implies cell migration [32]. The exact mechanism by which ANO1 modulates proliferation and migration of cancer cells is still unknown but Duvvuri and colleagues have recently reported that ANO1 might influence cell proliferation by activating the RAS–RAF–MEK–ERK1/2 pathway [33]. These data suggesting the involvement of ANO1 in cell proliferation and migration led us to hypothesize that ANO1 might be involved in airway epithelium repair.

Here, our main objectives were to study non-CF and CF airway epithelial repair and to assess ANO1 involvement in this process. We evaluated epithelial repair using proliferation and wound healing experiments in non-CF versus CF bronchial epithelial cells. We then compared ANO1 activity and expression between many non-CF and CF airway epithelial models. To assess the potential role for ANO1 in airway epithelial repair we tested the effect of ANO1 inhibition on proliferation