**Ineffective Correction of PPAR γ Signaling in Cystic Fibrosis Airway Epithelial Cells
Undergoing Repair**

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ABSTRACT

Peroxisome proliferator-activated receptor gamma (PPAR γ) represents a potential target to treat airway mucus hypersecretion in cystic fibrosis (CF). We aimed to determine if PPAR γ is altered in CF human airway epithelial cells (HAECs), if PPAR γ contributes to mucin expression and HAEC differentiation, and if PPAR γ ligand therapy corrects the CF phenotype. To this end, well-differentiated CF and NCF HAEC primary cultures were wounded to monitor the expression of key genes involved in PPAR γ activation and mucus homeostasis, and to evaluate the effect of a PPAR γ agonist, at different times of repair. Hydroxyprostaglandin dehydrogenase (HPGD) converts prostaglandin E2 to 15-keto PGE2 (15kPGE2), an endogenous PPAR γ ligand. Interestingly, PPAR γ and HPGD expression dramatically decreased in CF HAECs. These changes were accompanied by an increase in the expression of MUC5B. The correlation between PPAR γ and MUC5B was confirmed in an airway epithelial cell line after CFTR knock-down. Exposure of HAECs to 15kPGE2 did not correct the CF phenotype but revealed a defect in the process of basal cell (BC) differentiation. The HPGD/PPAR γ axis is deregulated in primary HAEC cultures from CF patients, which may impact the maturation of BCs to differentiated luminal cells. Importantly, PPAR γ therapy was inefficient in correcting the CF defect.

Key words: PPAR γ , ligand therapy, airway epithelium repair, mucus, human model, cystic fibrosis

1. Introduction

F508delCFTR is the most common mutation of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene causing cystic fibrosis (CF). CF is associated with defective Cl^- and HCO_3^- transport (Quinton, 2010). Dehydration of the secreted mucus layer, mucus hypersecretion, chronic infection and inflammation of airways are the main manifestations of CF (Cohen and prince, 2012). The continuous exposure to severe harmful stimuli places lungs at constant risk of injury and thereby, tissue repair is crucial for maintaining lung homeostasis (Puchelle et al., 2006; Wansleebe et al., 2013). The tracheobronchial airway epithelium is composed of basal (BCs), goblet/secretory (SCs) and ciliated cells (CCs) (Whitsett and Alenghat, 2015). It is well demonstrated that epithelium regeneration/repair is initiated by BC proliferation to repopulate the denuded injured area (Wansleebe et al., 2013). Once this state is reached, progenitor cells progressively mature to differentiated cells, exit the cell cycle, and return to their original state.

In CF, abnormal wound repair and hyperproliferation of BCs has been observed *in vivo* and in several models (Voynow et al., 2005; Hajj et al., 2007; Schiller et al., 2010; Trinh et al., 2012; Crespin et al., 2014; Itokazu et al., 2014). Thus, functional CFTR plays a critical role in regeneration of the airway epithelium, the repair of which is obviously insufficient to maintain lung functions in CF. One of the intracellular signaling pathways suggested to play a role in lung-related diseases is the lipid-responsive transcription factor peroxisome proliferator-activated receptor gamma ($\text{PPAR}\gamma$). In addition to its essential role in lung development (Huang et al., 2005), there is indication that CFTR deficiency causes intracellular changes in lipid metabolism that may be critical for the development of enhanced inflammation and susceptibility to infection (Ollero et al., 2004; Andersson et al., 2008; Perez et al., 2008; Dekkers et al., 2012). Furthermore, the inhibition of airway mucus hypersecretion

by PPAR γ agonists in other inflammatory diseases suggest that these molecules may represent drugs useful in the management of CF (Shen et al., 2012).

Here, we monitored the expression of genes involved in PPAR γ activation and mucus homeostasis in a circular wound model of well-differentiated CF and non-CF (NCF) human airway epithelial cells (HAECs) in primary cultures. The endogenous ligand of PPAR γ , 15-keto prostaglandin E2 (15kPGE2), is produced from PGE2 by the activity of hydroxyprostaglandin dehydrogenase (HPGD). We show that the expression of HPGD is dramatically decreased in CF HAECs. Our data also suggest that HPGD is involved in the PPAR γ -dependent control of the BC population during the repair process. Importantly, treatment with 15kPGE2 revealed altered sensitivity of CF repairing HAECs to PPAR γ activation and failure to correct the mucin overexpression phenotype.

2. Materials and Methods

2.1 Cell cultures

Well-differentiated primary cultures of NCF and CF HAECs were obtained from Epithelix (Epithelix Sàrl, Plan-les-Ouates, Switzerland). HAECs were differentiated on Transwell inserts at the air liquid interface (ALI) and cultured for 45–60 days before use, as previously reported (Wiszniewski et al., 2006). The characteristics of all primary cultures used in this study are summarized in Table 1. The human airway epithelial Calu-3 cell line was purchased from the American Type Culture Collection (Manassas, VA). CFTR knockdown was previously described (Bellec et al., 2015). More detailed information can be found in the online supplement.

2.2 Wound assay

Primary NCF and CF cells were mechanically wounded, as previously described (Crespin et al., 2014). Within hours, mechanical injury of the epithelium induced BC migration and proliferation until wound closure (WC), a time point that is usually reached about 48h after wounding (48hpW). From two days post WC (dpWC) on, the number of proliferating cells decreased back to basal levels. This allows to distinguish the “proliferation phase” and the “differentiation phase” of the repair process, as compared to the non-wounded (NW) condition. The undamaged HAECs out of the wound therefore contain the population of progenitor cells that initiate repair (Supplemental Figure 1). More detailed information can be found in the online supplement.

2.3 RNA Extraction and qPCR

The online supplement provides detailed information.

2.4 Histology and Mucin detection

The online supplementary provides detailed information.

2.5 Statistical Analysis

Data represent mean $\pm$ SEM. Two-way ANOVA test was used to analyze NCF/ CF gene expression during the time course of repair, independently. For each experiment on HAECs, the number of individual primary cultures (n) used from N donors is indicated. The Kruskal-Wallis test with the Dunn's multiple comparisons test correction or an Unpaired Student t-test was used to compare gene expression between samples within a given experimental condition, where appropriate, by using the Prism software (GraphPad Software, Inc., La Jolla, CA). Statistical significance was considered at $p < 0.05$. * is used to compare a wounded condition with NW in NCF cells; # is used to compare a wounded condition with NW in CF cells; \$ is used to compare two samples in a given experimental condition, as indicated in the text.

3. Results

3.1 Wound-induced repair of NCF and CF HAECs

Repair is a highly dynamic process involving heterogeneous cell populations. We measured the expression of BC (KRT5 and KRT14) and CC (DNAI1 and FOXJ1) markers in NW cultures and during the dynamic phases of repair at 24hpW, WC, and 10, 20 and 30 dpWC after wounding (Supplemental Figures 2a, 2c, 2e and 2g). The expression of KRT5 and KRT14 transiently increased at short time points of repair (24hpW or WC, hereafter referred as “proliferating phase”) and decreased back to basal levels from 10dpWC in both NCF and CF HAECs (Figures 1a and 1b). DNAI1 and FOXJ1 expression showed an opposite profile. As expected after injury, both CC markers were reduced during the “proliferating phase” but increased again with time of repair (10, 20 or 30 dpWC, hereafter referred as the “differentiating phase”) in NCF and CF HAECs (Figures 1c and d). No change in the expression of all markers was observed in the intact cells out of the injured area (Supplemental Figures 2b, 2d, 2f and 2h). These results indicate that in our HAEC model, wounding is associated with the transient loss of the DNAI1⁺/FOXJ1⁺ population of differentiated cells and the induction of KRT5⁺/KRT14⁺ BC proliferation at short time of repair. The expression of these BC markers returned to basal levels as early as 10dpWC, accounting for the ongoing re-differentiation at this time point, and re-expression of DNAI1/FOXJ1 markers. No difference in the expression of BC and CC markers was observed, however, between NCF and CF HAECs.

Next, we performed histological analysis of the re-differentiated airway epithelium in wounded NCF and CF HAEC cultures. NW and 30dpWC cultures were processed for paraffin sections, stained with Alcian blue to detect mucins. Both NCF and CF epithelia regenerated at least in terms of morphology 30dpWC (Figure 2a). Sticky mucus in repaired CF HAEC cultures was observed by the presence of intense blue staining that was difficult to wash off.

The relative proportion of BCs, CCs and SCs was similar in NCF and CF cells either under NW conditions or at 30dpWC (Figure 2b), although the variability in the unidentified (unknown) cell population was larger. These results indicate that regeneration after injury does not alter the global morphology of the airway epithelium nor the distribution of the three identifiable cell types after repair in CF primary cultures.

3.2 CF HAECs exhibit altered PPAR γ and mucin expression

PPAR γ has been involved in the control of mucus secretion and is known to be a key player in differentiation. As shown in Figure 3a, PPAR γ expression was not affected in NCF HAECs undergoing repair. However, PPAR γ expression was reduced in CF HAECs as compared to NCF cells throughout the repair process. Likewise, HPGD expression was strongly reduced in CF HAECs as compared to NCF cells (Figure 3b), suggesting that HPGD/PPAR γ signaling pathway is altered in CF HAECs.

These results prompted us to examine the expression of two major airway mucins, MUC5AC and MUC5B. MUC5AC expression transiently increased during the “proliferation phase” in both NCF and CF HAECs but was otherwise reduced in NW and “differentiating” CF HAECs as compared to NCF cultures (Figure 3c). MUC5B expression, however, was markedly enhanced in CF HAECs in all conditions (Figure 3d). Because bicarbonate production by airway epithelial cells is critical for mucus homeostasis (Quinton, 2010), we also monitored the expression of two carbonic anhydrase genes, CA2 and CA12, which are cytoplasmic and membrane bound, respectively. Although no difference was observed in the expression of CA12 in NCF and CF HAECs, CA2 expression was induced during the “proliferation phase” notably in CF cultures (Figures 3e and 3f). In summary, these gene expression profiles suggest an inverse proportionate relationship between HPGD/PPAR γ and MUC5B in CF HAECs.

Next, we silenced CFTR in Calu-3 cells (TSJB1-Cal-3 cells) by CRISPR-Cas9 gene editing (Bellec et al., 2015). Interestingly, CFTR knockdown was associated with decreased expression of PPAR γ (Figure 4a) and increased expression of MUC5B (Figure 4b). These results strengthen that there is a causal relationship between PPAR γ defect and MUC5B expression in CF airway epithelial cells.

3.3 PPAR γ contributes to the establishment of the differentiating signals in repairing HAECs

Actively proliferating BCs and their immediate daughters give rise to SCs and CCs during differentiation. The Notch pathway was reported to be critical to trigger SC lineages and Notch inhibition by DAPT shown to promote ciliogenesis (Rock et al., 2011). It was thus important to determine, in our model of HAEC repair, whether PPAR γ and Notch pathways intersect. To this end, we exposed repairing HAECs to the PPAR γ activator 15kPGE2 or to its irreversible antagonist GW9662. The efficiency of 15kPGE2 and GW9662 was first tested on the expression of the PPAR γ target gene PON2 (Supplemental Figure 3a). Similarly, the activity of the Notch pathway was confirmed in our cells by measuring the decreased expression of the Notch-regulated HES1 gene in the presence of DAPT (Supplemental Figure 3b). Exposure of repairing HAECs to 15kPGE2 or GW9662 had, however, no effect on HES1 expression, suggesting that PPAR γ does not intersect with Notch signaling throughout the repair process (Supplemental Figure 3c).

To identify the cell population targeted by PPAR γ in NCF HAECs, we measured the effects of 15kPGE2 and GW9662 on the relative changes of BC and CC cell marker expression throughout the repair process. These changes were compared to the control conditions at each time point. As shown in Figures 5a and 5b, significant changes were reached for 15kPGE2 that strongly inhibited KRT5 and KRT14 expression in “proliferating” BCs while inducing, by about 8 fold, the expression of KRT14 in “differentiating” HAECs.

No modulation, however, was observed for DNAI1⁺/FOXJ1⁺-expressing cells at all-time points (Figures 5c and 5d, respectively). These results indicate that BC populations (quiescent or active) are target of PPAR γ activity.

We next monitored the effects of PPAR γ modulators on PPAR γ and HPGD expression. Treatment of HAECs with 15kPGE2 or GW9662 had no effect on PPAR γ expression throughout the repair process (Figure 5e). In contrast, a potent induction by about 3 fold of HPGD expression was observed in NW cultures, and a tendency of 2.4 fold in “differentiating” cultures (Figure 5f). Thus, HPGD expression is sensitive to 15kPGE2-activated PPAR γ in differentiated HAECs, but not in the less mature “proliferating” BC cell population.

In summary, the data suggest that HPGD is part of a heterocellular system where its expression in more differentiated HAECs results in opposite PPAR γ activities in KRT5⁺/KRT14⁺ BCs according to their state of maturation.

3.4 PPAR γ agonist does not correct the CF phenotype during CF HAEC repair

PPAR γ is considered as a potential target to treat and reduce CF severity (Dekkers et al., 2012). In contrast to what was observed in NCF HAECs, 15kPGE2 decreased KRT5 (3.7 fold, Figure 6a) and DNA1 (2.4 fold, Figure 6c) expression in NW CF HAECs. KRT14 (1.65 fold, Figure 6b) and FOXJ1 (4 fold, Figure 6d) showed a tendency toward reduced expression in response to 15kPGE2. Under “proliferating” conditions, the decrease in gene expression for KRT5 and KRT14 remained detectable but not significant (Figures 6a-c) while DNAI1, surprisingly, was reduced (Figure 6d). 15kPGE2 had no effects on PPAR γ and HPGD expression (Figures 6e and 6f) in both conditions. In addition, PON2 expression in CF HAECs treated with 15kPGE2 was unaltered as compared to vehicle treatment (Figure 6g). Importantly, no effect of 15kPGE2 was detected on the expression of MUC5B (Figure 6h).

These results are consistent with the hypothesis that PPAR γ heterocellular signaling is defective in CF HAECs and that BCs are sequestered in a non-differentiating signal state.

4. Discussion

Our results describe the expression of genes related to the PPAR γ signaling pathway and mucus homeostasis during the repair process of primary HAEC cultures from CF patients. The use of primary cultures has appeared essential to investigate the transcriptional profile of native epithelia and therefore represent an excellent model to study the repair process in human (Pezzulo et al., 2010). In accordance with a recent study (Adam et al., 2015), the NCF and CF airway epithelia were morphologically similar after 30 days of re-differentiation in terms of their BC, SC and CC population. No difference in BC and CC marker gene expression was also observed. We, and others, have reported differences in the time course of repair of the CF airway epithelium (Trinh et al., 2012; Crespin et al., 2014; Adam et al., 2015). Therefore, some of the defects that might arise during CF repair are most likely at the level of the molecular pathways that fine-tune HAEC differentiation.

Notch family receptors and specific microRNAs regulate cell-fate specification in repairing airway epithelia (Marcet et al., 2011; Rock et al., 2011; Hogan et al., 2014; Mori et al., 2015). Although the Notch signaling pathway appeared unaltered between CF and NCF repairing HAECs, we found that PPAR γ and HPGD gene expression, and hence PPAR γ signaling, were strongly reduced in CF cultures. In NCF HAECs, exogenous addition of 15kPGE2 during the repair process revealed that BCs are targets of PPAR γ signaling. Interestingly, an opposite regulation of BC marker expression was observed between more mature (up-regulation in “differentiating” conditions) and less mature (down-regulation in “proliferating” conditions) BCs. This observation suggests that there are at least two populations of BCs with distinctive sensitivity to PPAR γ depending on their maturation state.

Heterogeneity of the BC population has been reported (Hong et al., 2004; Rock et al., 2009; Ghosh et al., 2011; Rock et al., 2011). By combining single-cell molecular analysis to lineage labelling and biophysical modelling, Watson and collaborators reported that the BC population comprised morphologically indistinguishable multipotent stem cells and committed precursors (Watson et al., 2015). In this context, we found a positive regulation of HPGD expression with 15kPGE2 in NCF differentiated HAECs, suggesting that HPGD expression is restricted to the more mature BC population or in already differentiated SCs or CCs. One possible scenario is that the more differentiated HPGD-expressing cells activate PPAR γ in less mature BCs, which in turn may contribute to regulate the proportion of active BCs exiting the cell cycle for further differentiation and/or trigger their return to their original state. In well-differentiated HAECs, the mutual regulation of PPAR γ and HPGD may provide a mechanism to maintain the KRT14⁺ BC pool in the absence of injury. It remains necessary, however, to identify the type of cells in which HPGD is expressed.

The reduced expression of HPGD and PPAR γ is likely to impact the differentiation process of CF HAECs. Indeed, CF cultures showed negative sensitivity to PPAR γ and lack of HPGD up-regulation by 15kPGE2 in NW cultures, in contrast to what was observed in NCF HAECs. These results are consistent with the hypothesis that the PPAR γ defect in CF HAECs sequesters BCs in a non-differentiating signal state. Although PPAR γ signaling is obviously not the sole mechanisms regulating HAEC fate during repair, our finding may provide an explanation to the hyperproliferative state and the delayed ciliogenesis previously reported for the CF airway epithelium (Voynow et al., 2005; Hajj et al., 2007; Crespin et al., 2014).

There is evidence that SCs are progenitors of CCs (Rawlins et al., 2009; Reynolds et al., 2012). According to Watson and collaborators, SCs are short-lived and give rise to the dominant CC population in well-differentiated airway epithelia (Watson et al., 2015). Here, we studied MUC5AC and MUC5B as SC markers because goblet cells are the predominant

secretory cell type in the human tracheobronchial airway, whereas Clara cells, which expresses *Scgb1a1*, are limited to the bronchioles (Wansleebe et al., 2013). We found that MUC5AC was transiently increased in both CF and NCF HAECs at short time points (24hpW and WC) of repair, and decreased back to basal levels at longer time points. This observation is in agreement with the scenario of BCs maturation giving rise to SCs that will later differentiate into CCs. Interestingly, MUC5AC expression was reduced in CF HAECs while MUC5B was up-regulated at all-time points of the repair process. Both MUC5AC and MUC5B are major components of the mucus layer in the human airways (Groneberg et al., 2002). MUC5B was shown to be overexpressed in a CF mouse model (Valque et al., 2011) and we confirmed here its induction after CFTR silencing in an airway epithelial cell line. Thus, our results in HAECs suggest that an enhanced MUC5B/MUC5AC gene expression ratio is a feature of CF.

Bicarbonate has been shown to be important for mucin expansion in the intestine and airways (Quinton, 2010; Ehre, Ridley and Thornton, 2014), and PPAR γ to have an essential role in reducing severity of the CF phenotype in intestines of CFTR^{-/-} mice through correction of HCO₃⁻ release (Harmon et al., 2010). Harmon and collaborators demonstrated that activation of the HPGD/PPAR γ signaling in CFTR^{-/-} mouse intestinal cells was associated with increased expression CA4 and CA2, increased HCO₃⁻ release by enterocytes and reduced mucus retention. Although we found that 15kPGE2 induced CA2 expression in uninjured NCF HAECs, this was not observed in CF cultures (Supplemental Figure 4a and b). Furthermore, the PPAR γ 's ligand did not correct the overexpression of MUC5B. One conclusion that can be drawn from these observations is that, unlike CFTR^{-/-} mouse intestinal cells, activation of the PPAR γ signaling does not correct the deregulated expression of mucus homeostasis-related genes in primary cultures of CF HAECs.

5. Conclusion

The HPGD/PPAR γ axis is deregulated in primary HAEC cultures from CF patients, which may impact the maturation of BCs to differentiated luminal cells and their MUC5AC/MUC5B expression. When activated by its ligand, PPAR γ forms a heterodimer with retinoic X receptor (RXR) and the complex PPAR γ /RXR binds to response elements of target genes. PPAR γ can also modulate gene expression by a transrepression mechanism that is independent of DNA binding but involves post-translational modifications, including phosphorylation, SUMOylation and ubiquitination (Dekkers et al, 2012). This complex regulation may explain the different sensitivity of BCs to 15kPGE2 according to their state of maturation/subtypes during the repair process. Evidence for altered lipid metabolism in CF has been extensively reported and reviewed (Al-Turkmani, Freedman and Lapasata, 2007; Becker et al., 2011). CFTR deficiency in airway epithelial cell lines and in F508delCFTR mouse models has been associated with up-regulation of transglutaminases 2, leading to defective PPAR γ SUMOylation and its sequestration in aggresomes (Maiuri et al., 2008; Luciani et al., 2009). It is therefore of clinical relevance to design novel approaches to bypass the PPAR γ defect that cannot be corrected in CF HAECs by PPAR γ ligand therapy in a context where inhibition of HPGD emerges as an effective means to promote tissue regeneration (FitzGerald, 2015).

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Appendix A. Supplementary data associated with this article can be found, in the online version.

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FIGURE LEGENDS

Figure 1: Gene expression profile during wound-induced repair in NCF and CF HAECs. The basal cell markers KRT5 and KRT14 (**a** and **b**) and the differentiated cell markers DNAI1 and FOXJ1 (**c** and **d**) were measured in non-wounded (NW) and in wounded epithelia during the “proliferation” (PP) and “differentiation” (DP) phases. Bars show mean $\pm$ SEM of $2^{-\Delta CT}$ values (n=5;11;18, N=5 for NCF and of n=4;8;10, N=4 for CF in **a**; n=5;12;18, N=6 for NCF and n=4;8;12, N=4 for CF in **b**; n=6;12;18, N=6 for NCF and n=3;8;11, N=4 for CF in **c**; n=6;12;17, N=5 for NCF and n=4;8;12, N=4 for CF in **d**) for each group considered, respectively. *p<0.05 in NCF with respect to non-wounded cultures (NW); #p<0.05 in CF with respect to NW.

Figure 2: Histology of NCF (top panels) and CF (bottom panels) airway epithelia before wounding (NW) and 30 days after wound closure (30dpWC). (**a**) Paraffin sections were stained with Alcian blue to reveal the mucus-secreting cells. Nuclei were counterstained with Nuclear Fast Red. The filters onto which HAECs are attached is visible at the basal side of the epithelia. Bar: 100 μ m. (**b**) Quantification of the different cell types identified according to their morphology in non-wounded (NW) and 30 days after wound closure (30dpWC) NCF and CF HAECs. Bars show mean $\pm$ SEM for each category the percentage from the total number of cells counted out of 2 independent NCF and CF cultures.

Figure 3: Expression profile of PPAR γ signalling and mucus homeostasis genes during wound-induced repair of NCF and CF HAECs. Bars show mean $\pm$ SEM of $2^{-\Delta CT}$ values for PPAR γ (**a**, n=11;21;18, N=11 for NCF and n=9;18;12, N=9 for CF), HPGD (**b**, n=11;20;18, N=11 for NCF and n=9;18;11, N=9 for CF), MUC5AC (**c**, n=6;12;17, N=6 for NCF and n=4;8;12, N=4 for CF), MUC5B (**d**, n=6;12;17, N=6 for NCF and n=4;7;12, N=4 for CF),

CA2 (**e**, n=6;11;17, N=6 for NCF and n=3;8;11, N=4 for CF) and CA12 (**f**, n=6;11;17, N=6 for NCF and n=4;7;12, N=4 for CF) gene expression in repairing HAECs for each group considered, respectively. *p<0.05 in NCF with respect to non-wounded cultures (NW); #p<0.05 in CF with respect to NW; §p<0.05 between NCF and CF. PP: proliferation phase; DP: differentiation phase.

Figure 4: Effect of CFTR knock-down on PPAR and MUC5B gene expression in Calu-3 cells. CFTR knockdown was achieved in Calu-3 cells by CRISPR-Cas9 gene editing (TSJB1 cells) and PPAR γ (**a**) and MUC5B (**b**) expression was determined as compared to a control Calu-3 cell line (CTL cells). PPAR γ expression was decreased in TSJB1 cells while MUC5B expression was increased. *p<0.05 in TSJB1 cells with respect to CTL cells (n=6).

Figure 5: Effect of PPAR γ modulation by 15kPGE2 and GW9662 on the expression of KRT5 HAEC markers during wound-induced repair of NCF HAECs. Bars show mean $\pm$ SEM (**a**, n=5;10;3, N=5 for DMSO; n=4;6;3, N=4 for 15kPGE2; n=5;10;3, N=5 for GW9662), KRT14 (**b**, n=5;10;3, N=5 for DMSO; n=4;6;3, N=4 for 15kPGE2; n=5;10;3, N=5 for GW9662), DNAI1 (**c**, n=5;7;3, N=5 for DMSO; n=5;7;3, N=4 for 15kPGE2; n=4;10;3, N=5 for GW9662), FOXJ1 (**d**, n=5;10;3, N=5 for DMSO; n=4;8;3, N=4 for 15kPGE2; n=5;10;3, N=5 for GW9662), PPAR γ (**e**, n=8;16;3, N=8 for DMSO; n=4;8;3, N=4 for 15kPGE2; n=8;16;3, N=8 for GW9662) and HPGD (**f**, n=8;16;3, N=8 for DMSO; n=4;8;3, N=4 for 15kPGE2; n=8;14;3, N=8 for GW9662) of the fold changes relative to the control condition (DMSO) for each group considered, respectively. NW: non-wounded, PP: proliferation phase; DP: differentiation phase. §p<0.05 between the treatments at indicated time-point.

537 **Figure 6:** Effect of PPAR γ modulation by 15kPGE2 on the expression of KRT5 (**a**), KRT14
538 (**b**), DNAI1 (**c**), FOXJ1 (**d**), PPAR γ (**e**) HPGD (**f**), PON2 (**g**) and MUC5B (**h**) during wound-
539 induced repair of CF HAECs. Bars show mean $\pm$ SEM of the fold changes (n=3, N=3) relative
540 to the control condition (DMSO) for each group considered. NW: non-wounded, PP:
541 proliferation phase; DP: differentiation phase. ^sp<0.05 between the treatments at indicated
542 time-point.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Table 1

Table 1: Clinical characteristics of Cystic Fibrosis and control Patients.

Donor	Gender	Age	CFTR Genotype	Source	Experiment
NCF1	M	66	WT	Bronchial	qPCR, Hist
NCF2	F	67	WT	Bronchial	qPCR
NCF3	M	61	WT	Bronchial	,qPCR
NCF4	F	46	WT	Bronchial	qPCR,DE
NCF5	M	61	WT	Bronchial	qPCR,DE
NCF6	F	54	WT	Bronchial	qPCR,DE
NCF7	M	78	WT	Bronchial	qPCR,DE
NCF8	F	40	WT	Nasal	qPCR,DE
NCF9	F	40	WT	Nasal	qPCR
NCF10	M	47	WT	Nasal	qPCR
NCF11	F	53	WT	Nasal	qPCR
NCF12	M	70	WT	Nasal	qPCR
NCF13	M	55	WT	Nasal	qPCR
NCF14	M	65	WT	Nasal	qPCR
NCF15	M	61	WT	Nasal	qPCR
NCF16	M	71	WT	Nasal	qPCR
NCF17	M	55	WT	Nasal	Hist
CF1	M	35	F508del	Bronchial	qPCR,DE
CF2	M	35	F508del	Bronchial	qPCR
CF3	F	25	F508del	Bronchial	qPCR,Hist,DE
CF4	F	29	F508del	Bronchial	qPCR
CF5	N/C	25	F508del	Bronchial	qPCR,Hist,DE
CF6	F	25	F508del	Bronchial	qPCR
CF7	M	23	F508del	Bronchial	qPCR
CF8	F	20	F508del	Bronchial	qPCR
CF9	F	40	F508del	Bronchial	qPCR
CF10	N/C	24	F508del	Nasal	qPCR
CF11	N/C	N/C	F508del	Nasal	qPCR

N/C: Non-Communicated; DE: drug exposure; Hist, histology