Cigarette smoke-induced oxidative stress in COPD
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Mitochondrial dysfunction increases pro-inflammatory cytokine production and impairs regeneration and glucocorticoid responsiveness in lung epithelium


Manuscript in preparation
Abstract

Noxious gases, including cigarette smoke, are the major risk factor for COPD, leading to chronic lung inflammation. Inhaled smoke first encounters the epithelial lining of the lungs, causing oxidative stress and mitochondrial dysfunction. We investigated whether a mitochondrial defect may contribute to increased lung epithelial pro-inflammatory responses, impaired epithelial repair and reduced glucocorticoid (GC) sensitivity as observed in COPD. We used wild-type alveolar epithelial cells A549 and mitochondrial DNA-depleted A549 cells (A549 Rho-0) and studied pro-inflammatory responses using (multiplex) ELISA, epithelial barrier function (electrical resistance) and wound repair using ECIS, in the presence and absence of the inhaled GC budesonide. We observed that A549 Rho-0 cells secrete higher levels of pro-inflammatory cytokines than wild-type A549 cells and display impaired recovery of epithelial integrity upon wound-healing. Furthermore, budesonide strongly suppressed cytokine production, and increased epithelial barrier function in wild-type cells, while A549 Rho-0 displayed reduced GC sensitivity compared to wild-type A549 cells with respect to the pro-inflammatory response and barrier function. This steroid unresponsiveness is likely mediated by glycolysis and the associated activation of PI3K signaling, as the specific PI3K inhibitor LY294002 restored GC sensitivity in A549 Rho-0. In conclusion, mitochondrial defects as observed in COPD patients may lead to increased lung epithelial pro-inflammatory responses, reduced regeneration and reduced GC responsiveness in lung epithelium, and thus contribute to the pathogenesis of COPD.

Abbreviations

COPD, Chronic Obstructive Pulmonary Disease; ECIS, Electric Cell substrate Impedance Sensing; Mn-SOD, Manganese Superoxide Dismutase; mtDNA, mitochondrial DNA; OXPHOS, Oxidative Phosphorylation; ROS, Reactive Oxygen Species.
Introduction

Chronic obstructive pulmonary disease (COPD) is a complex disease with increasing morbidity and mortality, characterized by irreversible airflow limitation and accelerated lung function decline. COPD is mainly caused by noxious environmental factors, including cigarette smoke, resulting in exaggerated lung inflammatory responses. Abnormal tissue repair and remodeling upon cigarette smoke-induced inflammation and damage is an important pathophysiologic feature of COPD, leading to fibrosis in the small airways and/or destruction of lung tissue (emphysema). Despite their broad anti-inflammatory effects, glucocorticoids (GC) provide relatively little therapeutic benefit in COPD. They reduce exacerbations, but do not effectively change the course of the disease nor the tissue damage that results from chronic airway inflammation (1, 2, 16, 22, 34). Furthermore, we recently observed that airway epithelial cells of COPD patients display reduced responsiveness to corticosteroids (15).

Inhaled cigarette smoke first encounters the epithelial lining of the lungs, where it induces oxidative stress. This inflicts lung epithelial cell death and damage, promoting the release of cytokines that attract and activate neutrophils (14). In addition, oxidative stress has been implicated in corticosteroid unresponsiveness (2, 6). Mitochondria are major intracellular targets of oxidative stress. Our previous findings have indicated that bronchial epithelial cells from COPD patients display mitochondrial abnormalities, with depletion of cristae and persistent mitochondrial damage. Similar mitochondrial changes were observed in epithelial cells that were exposed to cigarette smoke in vitro for 6 months (19). These abnormalities were accompanied by increased pro-inflammatory activity. However, it is currently unknown what the consequences of this mitochondrial dysfunction are and whether this may contribute to aberrant epithelial pro-inflammatory and repair responses in COPD. Of interest, Islam et al have recently shown that transfer of intact mitochondria by mesenchymal stem cells can contribute to lung tissue repair in a mouse model of lethal lung injury, suggesting that intact mitochondria may play be crucial for lung regeneration loss of mitochondrial function may impair this process (21). Furthermore, loss of mitochondrial function is known to lead to compensatory secondary metabolism, glycolysis, which has been implicated in glucocorticoid resistance in lymphoblastic leukemia (3, 20).

We hypothesize that mitochondrial dysfunction has important implications for epithelial responses, leading to increased pro-inflammatory activity, altered GC responsiveness and impaired epithelial barrier and repair responses. To address our hypothesis, we compared wild-type human alveolar A549 cells and A549 cells with depleted of functional mitochondria, A549 Rho-0. We show that A549 Rho-0 produce higher levels of pro-inflammatory cytokines, are less responsive to GC and display impaired wound healing responses compared to wild-type A549 cells.
Material and Methods

Cell culture
The wild-type alveolar type-II carcinoma cell A549 and mitochondria-depleted A549 Rho-0 cells were kindly provided by Dr. Lodovica Vergani (Padova University, Padova, Italy) and created by Ian Holt (Cambridge University, Cambridge, United Kingdom) by culturing A549 in addition of 50 ng/mL Ethidium Bromide for 8-12 passages. Cells were cultured in Dulbecco’s Modified Eagle’s medium (DMEM, Sigma, St. Louis, MO, (D6429-500ML) supplemented with MEM Amino Acids (50x) solution (Sigma, M7020-100ML), MEM Non-essential Amino Acid Solution, (Sigma, M7145-100ML), Vitamins, (Sigma, M6895-100ML) sodium pyruvate, Uridine 50 ng/ml (Sigma, cat. n. U-3003), 2,5 µg/ml amphotericin (Sigma-A2942-100ML), 25% foetal bovine serum (FBS; Hyclone, Logan, UT), 100 U/ml penicillin and 100 µg/ml streptomycin (Invitrogen (Gibco), Breda, The Netherlands) in uncoated T25 flasks. Before experimentation, cells were grown ~90% confluence and serum-deprived overnight.

Western blotting
Cell lysates were prepared and immunodetection was performed as described previously (17) using MitoProfile total OXPHOS antibody cocktail (Mitosciences, Eugene OR) anti-Mn-SOD (EMD Millipore Corporation, Billerica MA) and anti-GAPDH or anti-β-actin (Cell Signalling Technology, Danvers MA, USA) as loading control. Densitometry was performed using the gel-scan program QuantityOne.

ATP assay
Intracellular ATP levels of A549 were measured after ATP extraction. To extract ATP, cells were lysed using 0,5% TCA. Subsequently, TCA was neutralized with TE-buffer. ATP was measured with the luciferin–luciferase method (Enliten ATP assay system, Promega). Briefly, 100 µl sample was added to 50 µl of ATP assay mix and the luminescence was measured with a Luminoskan® Ascent microplate luminometer (Thermo Scientific, Waltham, USA) for 200ms per sample.

Cytokine levels
Cell-free culture supernatants (24 hours) were collected and analysed for CCL20 and CXCL8 using a duo-set ELISA assay (R&D Systems Europe, Abingdon, UK) or analysed for CXCL8 (IL-8), CCL20 (MIP-3α), CCL3 (MIP-1α), CCL4 (MIP-1β), G-CSF, IL-6, IL-12, CCL5 (RANTES), CXCL10 (IP-10), VEGF, IL-7, CCL2, IL-15 and EGF using a multiplex ELISA (Millipore, Billerica, MD) following manufacturer’s instructions.
Lactate assay
Lactate levels were measured in cell-free culture supernatants (24 hours) using a lactate assay kit (BioVision, Milpitas, USA) according to manufacturer’s protocol.

ECIS
Electrical resistance properties of confluent or wounded cells were measured using Electric Cell-substrate Impedance Sensing (ECIS, Applied Biophysics, Troy, NY, USA) as described previously (18). Upon inoculation in ECIS arrays, cells were incubated with/without 10⁻⁸M budesonide for 48 hours and resistance and capacitance were measured at 400Hz and 40kHz respectively. Cells were wounded by electroporation (5V, 40kHz, 30s) upon establishment of a confluent monolayer.

Confocal microscopy
Cells were stained with JC1, MitoTracker® DeepRed FM and Picogreen (Molecular Probes, Invitrogen) for 45 minutes in D-PBS (GIBCO, 14287-080) according to manufacture protocol. Subsequently, cells were washed twice with D-PBS and visualized using a Leica AOBS confocal microscope.

Statistical Analysis
The unpaired t-test followed by a Welch correction or a Mann-Whitney test was performed to evaluate differences A549 WT and Rho-0 cells and P=<0.05 was considered statistically significant (Figures 1 and 2). The Wilcoxon signed rank test (n=6) or a repeated measures ANOVA including a Bonferroni’s multiple comparison post-test (n=5) was used to evaluate differences within cell lines (Figures 3A and 3B) respectively. A two-way ANOVA was performed in addition using a Dunnett’s post-test when comparing time curves (Figures 4 and 5).

Results
Depletion of mitochondrial (mt) DNA and mitochondrial dysfunction in A549 Rho-0 compared to wild-type A549 cells
First, we assessed whether the mtDNA was successfully depleted in the A549 Rho-0 cells. We analyzed both wild-type A549 and A549 Rho-0 cell lines for mtDNA and the presence of mitochondrial bodies. Co-localization of mtDNA (Picogreen) and mitochondrial bodies (Mitotracker DeepRed) were clearly visible in the wild type A549 but not in the A549 Rho0, confirming the lack of mtDNA (Fig. 1a). JC1 staining, which detects changes in the mitochondrial membrane potential, showed a drastic decrease in membrane potential the A549 Rho-0 cells (Fig. 1b).
Figure 1. Mitochondrial (mt)DNA depletion and mitochondrial dysfunction in A549 Rho-0 cells. A549 wild-type and Rho-0 cells were grown to confluence and serum deprived overnight for 24 hrs. A) Both cell lines were stained with Picogreen (Green nuclei and mtDNA) and Mitotracker DeepRed. Co-localization of mtDNA and mitochondrial bodies is visible in the wild type A549, while no mtDNA is present in the A549 Rho0 cells. B) JC1 staining of A549 and A549 Rho0 cells. Mitochondrial membrane potential is lowered by depletion of mtDNA in the A549 Rho0 cells when compared to control cells. C) ATP levels (n=6-7) and D) lactate (n=3) were measured in cell-free culture supernatants and mean ± SEM are shown. E, F) Cell lysates were prepared and components essential for mitochondrial ATP production were detected by western blotting. A549 Rho-0 cells show significant lower levels of complex III and Complex IV but not for the ATPase subunit. Levels of Mn-SOD were not significantly altered (n=3). *=p<0.05 and **=p<0.01 between the indicated values as measured by an Welch corrected unpaired t-test.
Surprisingly, the intra-cellular concentrations of ATP did not differ between the wild-type A549 and the A549 Rho-0 cells, indicating an alternative pathway for ATP generation in A549 Rho0 cells than the mitochondrial-mediated oxidative phosphorylation (OXPHOS) process (Fig. 1c). Loss of mitochondrial function may lead to compensatory secondary metabolism, glycolysis, to produce ATP as well as lactate. To confirm a switch to glycolysis in the A549 Rho-0 cells, we analyzed lactate production. Indeed, lactate levels in the culture medium of A549 Rho-0 cells were significantly increased when compared to wild-type A549 cells (Fig. 1d). This was accompanied by a significant reduction in the expression specific components of the OXPHOS system, complex III subunit core 2 and complex IV subunit 3, further suggesting a switch to glycolysis (Fig. 1e), although the ATPase subunit α was not affected. In addition, we studied the expression of the mitochondrial anti-oxidant protein Mn-SOD, and observed a trend towards an increase in the A549 Rho-0 cells compared to the WT A549 cells, suggesting increased ROS production (Fig. 1f), although this failed to reach statistical significance.

Elevated pro-inflammatory cytokine/chemokine secretion in A549 Rho-0 cells

Next, we studied the effect of mitochondrial dysfunction on the pro-inflammatory cytokine response of lung epithelial cells. We measured a panel of pro-inflammatory cytokines/chemokines that have been associated with lung inflammation in COPD patients and/or show increased levels in COPD lungs (Table 1).

Table 1. Elevated cytokine/chemokine production and action in COPD

<table>
<thead>
<tr>
<th>Cytokine/chemokine</th>
<th>Effect in COPD</th>
<th>Mechanism in Disease</th>
<th>References</th>
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<tbody>
<tr>
<td>IL-6</td>
<td>Promotes disease</td>
<td>T-cell/B-cell Proliferator</td>
<td>(27, 38)</td>
</tr>
<tr>
<td>CXCL-8 (IL-8)</td>
<td>Promotes disease</td>
<td>Neutrophil Attractant and Activator</td>
<td>(4, 40)</td>
</tr>
<tr>
<td>CCL5 (RANTES)</td>
<td>Promotes disease</td>
<td>T-cell/eosinophil Attractant</td>
<td>(41)</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Promotes disease</td>
<td>Neutrophil Attractant</td>
<td>(29)</td>
</tr>
<tr>
<td>IL-12</td>
<td>Promotes disease</td>
<td>Th1 differentiator/Stimulates IFNγ</td>
<td>(12, 36, 39)</td>
</tr>
<tr>
<td>CXCL-10 (IP10)</td>
<td>Promotes disease</td>
<td>Monocytes/macrophages and T-cell Attractant</td>
<td>(13, 33, 39)</td>
</tr>
<tr>
<td>CCL3 (MIP1α)</td>
<td>Promotes disease</td>
<td>Neutrophil Attractant/Stimulates IL1/IL-6 release</td>
<td>(37)</td>
</tr>
<tr>
<td>CCL-4 (MIP1β)</td>
<td>Promotes disease</td>
<td>Neutrophil Attractant/Stimulates IL1/IL-6 release</td>
<td>(5, 31)</td>
</tr>
<tr>
<td>CCL20</td>
<td>Promotes disease</td>
<td>Strong Lymphocyte Attractant</td>
<td>(8, 32)</td>
</tr>
</tbody>
</table>

CCL20; Chemokine (C-C motif) Ligand 20; IL: Interleukin; IFNγ: Interferon gamma IL-1 Receptor Agonist; CXCL-10 (IP-10): Interferon gamma-induced Protein 10; CCL-3 or 4 (MIP-1α/β): Macrophage Inflammatory Protein 1α/β; CCL-5 (RANTES): Regulated on Activation, Normal T-cell Expressed and Secreted; Th2: Type 2 T-helper cell; VEGF: Vascular Endothelial Growth Factor; G-CSF: Granulocyte Colony-Stimulating Factor.
Of all the detected pro-inflammatory cytokines and chemokines, CXCL8 (IL-8), CCL20 (MIP-3α), CCL3 (MIP-1α), CCL4 (MIP-1β), G-CSF, IL-6 and IL-12, were significantly increased in Rho-0 compared to wild-type A549 cells, while a trend was observed for CCL5 (RANTES, \( p=0.0556 \)) and CXCL10 (IP-10, \( p=0.09 \)) (Fig. 2). These data suggest that mtDNA depletion results in an increased pro-inflammatory response in lung epithelial cells. We also found elevated levels of VEGF and IL-7 (Supplemental Figure 1a). Additionally, we found cytokines/chemokines that were unchanged between A549 WT and Rho-0, i.e. CCL2, IL-15 and EGF (Supplemental Figure 1b). INF-α, INF-γ, Eotaxin, IL-2, IL-13, FGF-basic, IL-1β, IL-4, IL-5, IL-10, IL-17, MIG, HGF and GM-CSF were hardly detectable in both cell types (data not shown).

**A549 Rho-0 cells show reduced GC responsiveness**

To investigate whether mitochondrial dysfunction may contribute to reduced GC sensitivity as observed in COPD (15), we studied the suppressive effect of budesonide on CXCL8 production in wild-type A549 and A549 Rho-0 cells. We observed that budesonide significantly reduced CXCL8 secretion by approximately 75-80% in the wild-type A549 cells. Of interest, budesonide failed to significantly suppress IL-8 secretion in the A549 Rho-0 cells (Fig. 3a). This suggests that a mitochondrial dysfunction triggers a pro-inflammatory response that is insensitive to corticosteroids.

Next, we studied whether the observed switch to glycolysis and activation of the associated PI3K/Akt signaling pathway is involved in the observed GC unresponsiveness in A549 Rho-0 cells, as PI3K has previously been implicated in GC unresponsiveness in COPD (28). We blocked PI3K/Akt activity, using the specific inhibitor LY294002 (10 μM). In both wild-type
A549 and A549 Rho-0 cells, we observed a marked reduction in IL-8 production when the PI3K pathway was blocked (Fig. 3b). Moreover, inhibition of the PI3K/Akt pathway restored corticosteroid sensitivity in A549 Rho-0 cells. In contrast to the absence of LY294002, budesonide significantly reduced CXCL8 production in A549 Rho0 cells in the presence of LY294002 (Fig. 3b). Thus, our data strongly suggest that the mitochondrial defect in A549 Rho-0 cells induces a PI3K/Akt-dependent GC insensitive pro-inflammatory response in lung epithelial cells.

**Figure 3.** A549 Rho-0 cells are less sensitive to budesonide than wild-type A549 cells, which is restored by blocking of PI3K/akt signaling. A549 wild-type and Rho-0 cells were grown to confluence, serum deprived overnight and incubated with/without budesonide (BUD, 10 nM) for 24 hours. A) CXCL8 was measured in cell-free supernatant (mean ± SEM, n=6). B) LY294002 (10µM) was added 30 min before the exposure to BUD and CXCL8 levels (mean ± SEM, n=5) are expressed as percentage of the levels without BUD. *=p<0.05, **=p<0.01 ***=p<0.001 between the indicated values.

**Reduced regenerative capacity in A549 Rho-0 cells**

In addition to pro-inflammatory responses, we studied whether mitochondrial dysfunction impacts on epithelial regeneration. We assessed the recovery of a confluent monolayer of A549 cells upon wounding by electroporation using the ECIS. Wild-type A549 were able to recover from this type of wounding within 4 hours, as observed by the return of resistance to values prior to wounding. This wound healing response was impaired in A549 Rho-0 cells, taking approximately 8 hours to recover, as reflected by stabilization of resistance values (Fig. 4). Moreover, resistance levels in A549 Rho-0 did not completely return to the values prior to wounding. This indicates that mtDNA depletion affects epithelial regenerative capacity. Together, A549 Rho-0 cells with defective mitochondria display abnormalities with respect
to pro-inflammatory responses, corticosteroid responsiveness as well as the capacity to regenerate upon wounding.

![Graph showing resistance over time](image)

**Figure 4.** A549 Rho-0 cells display reduced wound healing compared to wild-type A549 cells. A549 wild-type and Rho-0 cells were grown to confluence in ECIS arrays. Upon 2 days, cells were wounded by electroporation. Resistance was monitored for 22 hours at 40 Hz and levels were normalized to the values just prior to wounding (mean ± SEM, n=4). *** = p<0.001 between the indicated values as analyzed by 2-way ANOVA.

**Corticosteroid effect on barrier formation**

In addition to their suppressive effects on cytokines, we have previously reported that corticosteroids exert protective effects on epithelial barrier function (16). Therefore, we tested whether mitochondrial dysfunction also impairs the responsiveness to GC with respect to epithelial barrier function. Using ECIS, we measured low-frequency resistance and high-frequency capacitance as most sensitive parameters to measure epithelial cell-cell and cell-matrix contacts respectively, during cell growth in the presence and absence of budesonide (18). The stabilization of high-frequency capacitance at 20-30 hours indicates the formation of a confluent layer (Fig. 5a). After this, low-frequency resistance did not further increase, indicating that epithelial barrier function in A459 cells is mainly established by the formation of a monolayer, but not the formation of intracellular junctions. The presence of budesonide induced a two-fold increase in barrier function in wild-type A549 cells, which was observed for both high-frequency capacitance and low-frequency resistance (Fig. 5a and 5b). Although A549 Rho-0 cells showed a similar growth curve, A549 Rho-0 cells (Fig. 5a and 5b) were again less responsive to budesonide, as it did not significantly alter low-frequency resistance nor decreased high-frequency capacitance in these cells. Together, our results indicate that mitochondrial dysfunction impacts on epithelial pro-inflammatory responses, regeneration and corticosteroid responsiveness.
Figure 5. Budesonide improves barrier function in wild-type, but not Rho-0 A549 cells. A549 wild-type and Rho-0 cells were grown to confluence in ECIS arrays in the presence and absence of 10 nM budesonide (BUD) during 2 days. A) Capacitance was monitored at a frequency of 64 kHz using ECIS and normalized to the levels immediately after seeding (mean ± SEM, n=4). B) Resistance was monitored at a frequency of 40 Hz using ECIS and normalized to the levels immediately after seeding (mean ± SEM, n=4). *** = p<0.001 between the indicated values as analyzed by 2-way ANOVA.
Discussion

We show for the first time that alveolar epithelial cells lacking functional mitochondria display increased production of pro-inflammatory cytokines and loss of responsiveness of CXCL8 production to GC. This is accompanied by increased pro-inflammatory responses, impaired regenerative responses and reduced responsiveness of epithelial barrier function and pro-inflammatory cytokine production to the GC budesonide, which may be mediated by activation of the glycolysis (Fig. 6). While we observed higher levels of lactate produced by A549 cells with dysfunctional mitochondria, inhibition of the glycolysis-associated PI3K pathway reduced pro-inflammatory cytokine production and restored GC sensitivity.

We previously observed long-term cigarette smoke-exposed bronchial epithelial cells display mitochondrial abnormalities, and that these are accompanied by increased production of the pro-inflammatory cytokines CXCL8 and IL-6 (19). Moreover, cultured bronchial epithelial cells from COPD patients also show mitochondrial abnormalities (19) and produce higher levels of CXCL8 at baseline than epithelial cells from control donors (35). We now show that mitochondrial dysfunction leads to increased levels of CXCL8, CCL20, G-CSF, CCL3 and CCL4, IL-6 and IL-12, all of which are increased in lungs of COPD patients (4, 5, 8, 12, 27, 29, 31, 32, 36–40). Our current and previous (19) findings may also explain why bronchial epithelial cells from COPD patients are less responsive to corticosteroids with respect to both cytokine production as well as barrier function, as observed previously (18).

Our current findings are limited to the use of a cell line, since the depletion of mtDNA for creating Rho-0 cells requires long-term exposure to a low dose of ethidium bromide. In future studies, it will be of interest to test the effect of mitochondrial function blockers/uncouplers on pro-inflammatory responses in primary epithelial cells.

When depleted of mtDNA, cells are not able to perform normal electron transport or ATP synthesis and rely on ATP derived from glycolysis, where glucose is metabolized for survival and growth to produce lactate (7). This metabolic switch is dependent on the activation of PI3K signaling, as demonstrated in naive T cells and cancer cells (9, 10). Of interest, it has previously been reported that GC resistance in T-lineage acute lymphoblastic leukemia is associated with the upregulation of glycolysis and activation of PI3K/AKT/mTOR signaling (3, 20). We observed a switch to glycolysis in Rho-0 cells, as indicated by increased lactate production and reduced expression of specific OXPHOS components. Furthermore, we found that inhibition of PI3K reversed the GC insensitivity of CXCL8 production in A549 Rho-0 cells. PI3K inhibition itself also significantly reduced CXCL8 production, and the GC unresponsiveness of A549 Rho-0 cells could thus be a consequence of PI3K activity being insensitive to GC, resulting in GC-insensitive CXCL8 production. Alternatively, PI3K activity is known to reduce GC responsiveness, e.g. by mechanisms involving post-translational histone deacetylase 2 (HDAC2) modifications and proteasomal degradation of HDAC2, leading to altered acetylation of the GC receptor and/or histones within the promoter regions of
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pro-inflammatory genes (22, 23, 30). We have previously demonstrated that IL-17 reduces GC responsiveness of CXCL8 production in airway epithelial cells in a PI3K and HDAC2-dependent manner (24). Similar mechanisms may be involved in GC unresponsiveness upon mtDNA depletion, although further studies will be required to establish whether mitochondrial dysfunction also leads to a reduction in HDAC expression. It is currently unknown whether similar mechanisms may be involved in the inability of GC to improve the integrity of A549 Rho-0 monolayers. Since GC fail to efficiently suppress inflammation in the majority of COPD patients and to prevent the loss of alveolar tissue in emphysema, it will be of interest to elucidate the precise mechanisms of GC unresponsiveness in mitochondria-depleted epithelial cells.

Figure 6. Proposed mechanism of reduced glucocorticoid (GC) insensitivity upon mitochondrial dysfunction. Lung cells with mitochondrial dysfunction are more prone to pro-inflammatory responses through the PI3K/Akt-pathway upon a switch to glycolysis, leading to reduced GC sensitivity of cytokine production as well as barrier function.

In addition to the changes in GC responsiveness, our findings may have additional implications for COPD, as aberrant tissue repair responses are thought to contribute the pathogenesis of the disease. We observed that mitochondrial depletion impairs the ability of epithelial cells to close wounds. Although we did not further study the mechanisms by
which functionally intact mitochondria may promote epithelial regeneration or modulate epithelial adhesion, it is conceivable that migration of epithelial cells to cover the wounded area requires active energy metabolism. Of interest, lung ageing is also associated with mitochondrial dysfunction and impaired repair capacity of lung epithelial cells, fibroblasts and stem cells, leading to emphysema-like features (11, 19, 25, 26). It will therefore be of interest to study the effect of mitochondrial function blockers on the regenerative response of primary bronchial epithelial cells in future studies.

In conclusion, our data indicate that mitochondrial dysfunction leads to increased pro-inflammatory activity, inefficient wound healing and reduced responsiveness to GCs in alveolar epithelium. Our results suggest that novel strategies towards improved mitochondrial function may be promising in COPD. Furthermore, restoration of steroid responsiveness, e.g. by means of pharmacological inhibition of the PI3K pathway, may increase the suppressive effects of GC on lung inflammation and improve the integrity of lung epithelial cells in COPD.

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References


Supplemental Information
Supplemental Figure 1. Cytokine and chemokine secretion in A549 Rho-0 cells. A549 wild-type and Rho-0 cells were grown to confluence and serum deprived overnight. A) VEGF, IL-7. B) CCL2, IL-15 and EGF were measured in cell-free supernatants (mean ± SEM, n=5) *p<0.05 between the indicated values as analysed by a Mann-Whitney test.