Glucocorticoids increase C/EBPβ activity in the lung epithelium via phosphorylation

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Abstract

Glucocorticoids are widely prescribed anti-inflammatory drugs used for the treatment of many inflammatory lung disorders. However, much still remains unknown about their molecular mechanisms of action. We have previously shown that glucocorticoid-induced transcription in the lung epithelial cell line NCI-H441 is mediated via C/EBP sites in the promoters of target genes, and is likely to involve the transcription factors C/EBPβ and C/EBPδ. Here, we report that C/EBPβ is the most active C/EBP-factor in both human and mouse lung epithelium and that glucocorticoids induce DNA binding of C/EBPβ in cultured primary mouse lung epithelial cells. Mechanistic studies in H441 cells revealed that glucocorticoids, acting via the glucocorticoid receptor, increase C/EBPβ binding starting 10 min after stimulation. The mechanism is independent of de novo protein synthesis and involves phosphorylation of C/EBPβ at Thr²³⁵. Together this shows that glucocorticoids increase DNA-binding activity of C/EBPβ via post-translational mechanism(s) involving phosphorylation.

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Glucocorticoids are among the most widely prescribed anti-inflammatory drugs, frequently and effectively used in treatment of various inflammatory lung disorders, such as asthma. However, glucocorticoid treatment of the inflammatory lung disorder chronic obstructive pulmonary disease (COPD) is less effective [1–3], suggesting that glucocorticoid signaling in the lung is not yet fully understood. During normal conditions, glucocorticoids are vital for lung development and maturation. Mice lacking the glucocorticoid receptor exhibit highly immature lungs with hyperproliferation of the epithelium and they die shortly after birth due to respiratory failure [4]. Glucocorticoids regulate transcription via the glucocorticoid receptor which upon activation translocates to the nucleus, binds to DNA, and activates or represses gene transcription. Additionally, the glucocorticoid receptor controls transcription via DNA independent mechanisms, such as direct protein–protein interactions [5]. These mechanisms seem to be of particular importance during lung development, shown by transgenic mice carrying a mutated form of the glucocorticoid receptor [6]. Despite this mutated receptor being incapable of binding DNA, the mice are viable and do not show any of the respiratory problems seen in the glucocorticoid receptor knockout mice. Moreover, several genes regulated by glucocorticoids in lung, such as those encoding the surfactant proteins (SP) and the Clara cell secretory protein (CC16), lack functional binding sites for the glucocorticoid receptor in their promoters [7,8]. Together this suggests mechanisms activating transcription independent of glucocorticoid receptor binding to
The present study investigates the mechanism by which glucocorticoids regulate transcription in lung epithelium via C/EBPs. We report here that C/EBPβ is the most active C/EBP-factor in both human and mouse lung epithelium and that glucocorticoids induce DNA binding of C/EBPβ in cultured primary mouse lung epithelial cells. Mechanistic studies in the human lung epithelial cell line NCI-H441 revealed that glucocorticoids enhance binding of C/EBPβ and C/EBPδ after 10 min of treatment. The mechanism is mediated via the glucocorticoid receptor and is partially serum-dependent but independent of de novo protein synthesis and involves phosphorylation of C/EBPβ at Thr535. This demonstrates that glucocorticoids increase the DNA-binding activity of C/EBPβ in the lung epithelium via a post-translational mechanism(s) involving phosphorylation, representing a novel signal transduction pathway for glucocorticoids.

Materials and methods

Chemicals. All chemicals used were obtained from Sigma (LO), unless otherwise stated. Brush biopsies. Brush biopsies from two healthy volunteers were collected by fiberoptic bronchoscopy as previously described [10]. Approximately one million cells were obtained of which >90% are epithelial [11]. Nuclear extracts were prepared as previously described [12]. Cells were fixed in 4% paraformaldehyde with 1% Triton X-100 at 4°C for 15 min and antigens were revealed by boiling in citrate buffer (pH 6.0) for 30 min, followed by incubation with 5% horse serum in phosphate-buffered saline (PBS) containing 0.1% Triton for 4 h at 4°C to block unspecific binding of antibody. After overnight incubation at 4°C with C/EBPα and C/EBPδ primary antibodies (diluted 1:100 in PBS with 10% horse serum) and washing in PBS (with the last washes in PBS containing 0.3% Triton), antibody-antigen complexes were detected by using a FITC conjugated secondary antibody (diluted 1:100 in PBS and 2% horse serum for 1 h). Cells were counterstained with DAPI, mounted with Vectashield (Vector, UK), and viewed under a Zeiss Axiosplan 2 fluorescent microscope with filters for FITC and DAPI.

Western blotting. Cellular proteins (36 μg) were resolved on 12% SDS–PAGE gels and proteins were transferred overnight at 4°C to Protran nitrocellulose membrane (Schleicher and Schuell, Germany). After blocking in Tris-buffered saline (TBS) with 0.1% Tween and 5% milk for 1.5 h, the membrane was incubated with primary antibody overnight diluted 1:10,000 in TBS with 5% non-fat dry milk. As positive controls, C/EBPα-factors were overexpressed by transient transfection of COS-1 cells as previously described [13] using C/EBPα, C/EBPβ, and C/EBPδ plasmids described in [10]. Antibodies used were: anti-C/EBPα (sc-150x), anti-C/EBPβ (sc-636x; both from Santa Cruz Biotechnology, CA), anti-phospho-Thr235-C/EBPβ, anti-ERK, and anti-dual-phospho-ERK (all three from Cell Signaling Technology, MA). After washing in TBS, primary antibodies were detected with anti-rabbit peroxidase-labeled antibody diluted 1:10,000 in PBS with 0.1% Tween and 5% milk for 1.5 h. After washing, the secondary antibody was detected using ECL Western Blotting detection reagent (Amersham Biosciences, UK).

Isolation and culture of primary lung cells. Isolation of mouse C57BL/6 lung cells was carried out as previously described [14]. Briefly, lungs were perfused with PBS before removal and alveolar macrophages were removed via airway lavage. The lungs were treated with trypsin via intra tracheal instillation, mechanically minced and filtered to give a single cell suspension. Cells were pelleted by centrifugation and seeded in standard Petri dishes to allow non-epithelial cells to attach. After 2 h incubation, non-adherent cells were seeded onto cell culture plates (BD Falcon, CA) and treated with dexamethasone (dex) for 3 h. Cells were fixed in 4% paraformaldehyde with 1% Triton X-100 at 4°C for 15 min and antigens were revealed by boiling in citrate buffer (pH 6.0) for 30 min, followed by incubation with 5% horse serum in phosphate-buffered saline (PBS) containing 0.1% Triton for 4 h at 4°C to block unspecific binding of antibody. After overnight incubation at 4°C with C/EBPβ and C/EBPδ primary antibodies (diluted 1:100 in PBS with 10% horse serum) and washing in PBS (with the last washes in PBS containing 0.3% Triton), antibody–antigen complexes were detected by using a FITC conjugated secondary antibody (diluted 1:100 in PBS and 2% horse serum for 1 h). Cells were counterstained with DAPI, mounted with Vectashield (Vector, UK), and viewed under a Zeiss Axiosplan 2 fluorescent microscope with filters for FITC and DAPI.

Electronic mobility shift assays. A double-stranded synthetic oligonucleotide harboring a consensus C/EBP-binding site (underlined; 5′-CGG GAT CCA TTG CGC AAT GGA TCC-3′) was end-labeled using γ[32P]ATP and T4 polynucleotide kinase (Amersham Biosciences, UK). Electrophoretic mobility shift assays were performed as previously described [12] using 3–7 μg of nuclear proteins. With this condition, 1 μl of polyclonal antibodies, anti-C/EBPα (sc-61x), anti-C/EBPβ (sc-150x) or anti-C/EBPδ (sc-636x; all from Santa Cruz Biotechnology, CA) were added. Protein–DNA complexes were resolved on pre-electrophoresed non-denaturing 5% polyacrylamide gels, vacuum-dried, and exposed to autoradiographic film or quantified with a phosphoimager using FUJIX phosphoimager plates (Fuji, Japan) and a Molecular Imager FX (Bio-Rad, CA).

Immunofluorescence. NCI-H441 cells were seeded (4.7 ×104 cells/cm²) onto culture slides (BD Falcon, CA) and treated with dexamethasone (DEX, 1 μM) for 2 h. Cells were fixed in 4% paraformaldehyde with 1% Triton X-100 at 4°C for 15 min and antigens were revealed by boiling in citrate buffer (pH 6.0) for 30 min, followed by incubation with 5% horse serum in phosphate-buffered saline (PBS) containing 0.1% Triton for 4 h at 4°C to block unspecific binding of antibody. After overnight incubation at 4°C with C/EBPβ and C/EBPδ primary antibodies (diluted 1:100 in PBS with 10% horse serum) and washing in PBS (with the last washes in PBS containing 0.3% Triton), antibody–antigen complexes were detected by using a FITC conjugated secondary antibody (diluted 1:100 in PBS and 2% horse serum for 1 h). Cells were counterstained with DAPI, mounted with Vectashield (Vector, UK), and viewed under a Zeiss Axiosplan 2 fluorescent microscope with filters for FITC and DAPI.
Results

C/EBPβ is the most active C/EBP-factor in the human airway epithelium in vivo

We have previously found that in the highly differentiated human lung epithelial cell line NCI-H441, the CC16 and CYP2B1 genes are induced by glucocorticoids via C/EBP-binding sites in their promoters and that this effect is likely to involve C/EBPβ and δ [9].

To investigate the binding profile of C/EBPs in the human lung epithelium, airway epithelial cells were obtained from brush biopsies of two volunteers during bronchoscopy (Fig. 1). C/EBP-binding activity in the cells was studied by electrophoretic mobility shift assay (EMSA) using an oligonucleotide harboring a C/EBP-consensus-binding site. Inclusion of C/EBPα, C/EBPβ, and C/EBPδ antibodies revealed that the majority of the shift was composed of C/EBPβ (Fig. 1, lanes 3 and 7). Some C/EBPδ binding was also seen (Fig. 1, lanes 4 and 8), but no C/EBPα-binding activity could be detected (Fig. 1, lanes 2 and 6). Thus, C/EBPβ is the dominant C/EBP DNA-binding factor in the human airway epithelium. This binding pattern is similar to the binding pattern seen in NCI-H441 cells, where DNA binding of C/EBPβ and C/EBPδ, but not C/EBPα, is detected [9]. This validates the use of NCI-H441 cells as a model for further studies.

Glucocorticoids induce DNA binding of C/EBP within 10 min in a dose-dependent manner

To investigate the dose–response relationships of the effects of glucocorticoids on C/EBP DNA binding, NCI-H441 cells were incubated with 0–1000 nM of the synthetic glucocorticoid homologue dexamethasone (dex) for 3 h (Fig. 2A). Nuclear extracts were analyzed by EMSA and exposed to film. The induction of DNA binding was dose dependent between 0.1 and 1000 nM. The most marked increase was seen from 0.1 to 10 nM (Fig. 2A), which corresponds well to the \( K_d \) of dex for the glucocorticoid receptor (1.8 nM). To investigate the kinetics of the glucocorticoid-induced increase in C/EBP-binding activity, NCI-H441 cells were treated with 12 nM dex between 10 min and 24 h, and the C/EBP DNA binding was measured by EMSA as above. The DNA binding increased within 10 min after...
Dexamethasone induction of CIEBP DNA binding is mediated through the glucocorticoid receptor independently of de novo protein synthesis

To investigate whether glucocorticoids act on CIEBPs via the glucocorticoid receptor, competition experiments with the glucocorticoid receptor antagonist RU486 were performed (Fig. 3A). When RU486 was added to NCI-H441 cells in a 200-fold excess over dex, RU486 completely blocked the dex-induced increase in DNA binding of the CIEBPs (Fig. 3A, lane 4). This demonstrates that glucocorticoid activation of CIEBPs occurs via the glucocorticoid receptor.

When levels of CIEBPβ and CIEBPδ were analyzed with Western blotting at 20 min–24 h after addition of glucocorticoids, protein levels were stable (Figs. 3C and D, lanes 2–5, respectively), indicating that the increased binding of CIEBPs is not a result of increased protein levels. To investigate if de novo protein synthesis is required for increased CIEBP binding, NCI-H441 cells were pretreated with cyclohexamide (CHX), an inhibitor of protein translation, for 1 h prior to dex stimulation. CHX treatment blocked protein synthesis to 95% (data not shown), but did not affect the increase in CIEBP DNA binding induced by glucocorticoid treatment (Fig. 3B, lane 3 and 4). CHX alone did not affect CIEBP binding (Fig. 3B, lanes 1 and 3) and CIEBP protein levels remained stable (Figs. 3C and D, lanes 6–9), confirming that the increased DNA binding is not a result of, or dependent on, de novo protein synthesis.

Dexamethasone does not influence intracellular translocation of CIEBPs

The fact that increased DNA binding occurs within 10 min and was independent of de novo protein synthesis raises the possibility that glucocorticoid treatment influences CIEBPs via a non-transcriptional/translation mechanism such as a post-translational modification. As previously described, post-translational modifications can increase the DNA-binding activity and/or the nuclear translocation of CIEBPs [15–17]. Since nothing so far is known about the influence of glucocorticoids on CIEBP-translocation events, CIEBPβ and CIEBPδ protein levels in nuclear, cytosolic, and whole cell extracts were compared after 30 min and 3 h of dex treatment. Both CIEBPβ (Fig. 4) and CIEBPδ (data not shown) were present in all extracts, but there were no changes in protein levels between the extracts after dex treatment. Nor were we able to detect intracellular or nuclear translocation of CIEBPβ or CIEBPδ after glucocorticoid treatment using immunofluorescence microscopy (data not shown).

Fig. 3. Dexamethasone induction of CIEBP DNA binding is mediated through the glucocorticoid receptor independently of de novo protein synthesis. (A) Nuclear extracts from NCI-H441 cells untreated or treated with 10 nM dex and 2 µM RU486 for 3 h were analyzed by EMSA using a CIEBP-consensus oligonucleotide probe. (B) NCI-H441 cells were pretreated for 1 h with 5 mM cyclohexamide (CHX), to inhibit protein synthesis, prior to treatment with 12 nM dex for the time points indicated. Nuclear extracts were analyzed for CIEBP binding by EMSA using a CIEBP-consensus oligonucleotide probe. (C,D) Nuclear extracts from NCI-H441 cells untreated or treated with dex and CHX for the indicated times was analyzed by Western blotting. Expression of CIEBPs was detected by antibodies against CIEBPβ (C) or CIEBPδ (D). First lanes contain overexpressed CIEBPβ or CIEBPδ proteins as positive controls, prepared as described in [13].

Fig. 4. Glucocorticoids do not induce nuclear translocation of CIEBPβ. Nuclear extracts (lanes 1–3), cytosolic extracts (lanes 4–6), and whole cell extracts (lanes 7–9) from NCI-H441 cells, untreated or treated with 12 nM dex for 30 min or 3 h, were analyzed by Western blot analysis with antibodies against CIEBPβ.
Glucocorticoids induce phosphorylation of C/EBP\textsubscript{b} on Thr\textsuperscript{235}

ERK1/2, a Ser/Thr MAP kinase, is known to be a key regulator of C/EBP\textsubscript{b} activation [18–20]. By phosphorylating Thr\textsuperscript{235} on C/EBP\textsubscript{b}, ERK1/2 enhances the ability of C/EBP\textsubscript{b} to bind DNA and induce transcription. By Western blotting, using an antibody that specifically recognizes Thr\textsuperscript{235}-phosphorylated C/EBP\textsubscript{b} [20], Thr\textsuperscript{235}-phosphorylation of the larger of the two C/EBP\textsubscript{b} forms was observed within 30 min of glucocorticoid treatment and even more after 3 h (Fig. 5A, panel I). The smaller isoform appeared to be constitutively phosphorylated at Thr\textsuperscript{235}. C/EBP\textsubscript{b} usually appears in two or more bands on Western blots, presumably because of differential post-translational modifications [21]. Total levels of C/EBP\textsubscript{b} remained unchanged (Fig. 5A, panel II). As the Thr\textsuperscript{235} phosphorylation is known to affect the DNA-binding activity of C/EBP\textsubscript{b} it could underlie the glucocorticoid-induced increase in DNA binding. We therefore used the chemical enzymatic inhibitor U0126 at 10 nM to inhibit MEK1/2, an upstream regulator of ERK1/2. However, blockade of MEK1/2 did not alter the ability of dex to enhance C/EBP DNA-binding activity (Fig. 5B). Also, dual-phospho ERK antibody and ERK antibody revealed that glucocorticoid treatment neither activated, i.e. dual-phosphorylated ERK1/2 (Fig. 5A, panel III), nor changed the total protein levels of ERK1/2 (Fig. 5A, panel IV). This suggests that glucocorticoids influence the phosphorylation status of Thr\textsuperscript{235} on C/EBP\textsubscript{b}, a previously described ERK1/2 phosphorylation site, independently of the classic MEK1/2–ERK1/2 pathway.

This puzzling observation led us to test additional inhibitors directed against other pathways previously described to affect C/EBP activity. NCI-H441 cells were pretreated with inhibitors for 30 min and then treated with dex for 30 min–3 h. Inhibitors used were SB203580 at 10 nM to inhibit p38 kinase (data not shown), LY294002 at 50 nM to inhibit PI3K (data not shown), staurosporine at 20 nM to block PKC, PKA, PKG, and CaM kinase signaling pathways (data not shown), or peroxiorthovanadate at 50 \mu M, a broad-spectrum phosphatase inhibitor (Fig. 5C). None of these inhibitors altered the ability of dex to enhance C/EBP DNA-binding activity, suggesting that glucocorticoids do not induce C/EBP DNA binding through any of these previously described pathways. However, all inhibitors used did increase or reduce the basal DNA-binding activity of C/EBPs, as expected from previous publications [17], indicating that the inhibitors were all active and confirming that phosphorylations are important in modulating C/EBP function (data not shown). In addition, depletion of serum from the cell culture medium caused a delayed response of glucocorticoids such that increased C/EBP binding was not seen until after 3 h (data not shown). This, together with the fact that serum has been shown to induce C/EBP\textsubscript{b} activity via MAP kinase pathways [22], suggests that serum is needed for the initial phase of the glucocorticoid response, further indicating that glucocorticoid action on C/EBPs is mediated via phosphorylation-dependent signaling.

Even though our data do not conclusively demonstrate that phosphorylation of Thr\textsuperscript{235} mediates the increase in C/EBP\textsubscript{b} DNA binding, the increased phosphorylation of Thr\textsuperscript{235}, together with the dependence on serum, indicates that phosphorylation signaling is involved in mediating the glucocorticoid-induced effects on C/EBP\textsubscript{b} activity.

![Fig. 5. Glucocorticoids induce phosphorylation of C/EBP\textsubscript{b} on Thr\textsuperscript{235} independent of MEK1/2 or phosphatase-dependent pathways. (A) Nuclear extracts from NCI-H441 cells untreated or treated with 12 nM dex for 30 min or 3 h were analyzed by Western blotting for levels of C/EBP\textsubscript{b} phosphorylated on Thr\textsuperscript{235}, a known target for ERK1/2 (panel I), total C/EBP\textsubscript{b} (panel II), activated (dual-phospho) ERK1/2 (panel III), and total ERK1/2 (panel IV). (B,C) Nuclear extracts from NCI-H441 cells untreated or treated for 30 min with 10 nM U0126 to inhibit MEK1/2 (B), or with 50 \mu M peroxiorthovanadate, a phosphatase inhibitor (C), prior to 30 min or 3 h dex (12 nM) stimulation, were analyzed for C/EBP binding by EMSA using a C/EBP-consensus oligonucleotide probe.](image-url)
Glucocorticoids induce C/EBP DNA binding in primary mouse lung epithelial cells

On the basis of the lung epithelial cell line NCI-H441 having a similar C/EBP profile as human airway epithelial cells with C/EBPβ being the dominant C/EBP DNA-binding factor, we used this cell line as our model and found that glucocorticoids increase the DNA-binding activity of C/EBPβ. To investigate the effects of glucocorticoids on C/EBP activity in the primary lung epithelium, epithelial cells from C57BL/6 mice were isolated and cultured in vitro. The primary lung cells were cultured for 42 h and then incubated with dex for 30 min and 3 h with Western blotting using a C/EBPβ antibody and the levels were unchanged after treatment with dex (Fig. 6A, lanes 2 and 3). EMSA experiments revealed increased C/EBP DNA binding after 3 h (Fig. 6B, lane 3). C/EBPβ antibody was able to super-shift the entire shift (Fig. 6B, lanes 4–6) and the C/EBPδ antibody super-shifted a smaller fraction (Fig. 6B, lanes 7–9), revealing that C/EBPβ was the predominant DNA-binding factor in the mouse lung epithelium as well (Fig. 6B, lanes 4–6). In agreement with the results from the NCI-H441 cells [9], binding of C/EBPβ was further increased after 24 h treatment with dex without affecting the protein levels (data not shown). This demonstrates that the binding profile and glucocorticoid responses of C/EBPs in vivo is similar to that seen in NCI-H441 cells, with glucocorticoids inducing DNA binding of predominantly C/EBPβ in the lung epithelium without affecting protein levels.

Discussion

In this study, we report that glucocorticoid treatment increases the DNA-binding affinity of the transcription factor C/EBPβ in the highly differentiated human lung epithelial cell line NCI-H441 as well as in primary mouse lung epithelial cells. Investigations of the underlying mechanisms revealed that this occurs via a rapid post-translational modification involving phosphorylation, however, it is independent of the signaling pathways previously shown to affect CEBP activity. We also report that C/EBPβ is the predominant C/EBP DNA-binding factor in both human and mouse airway epithelium. The fact that the same DNA-binding profile of C/EBPs was seen in epithelial cells from both human and mouse, and that treatment with glucocorticoids increased the binding activity of C/EBPβ in mouse primary lung epithelial cells comparable to the results in NCI-H441 cells, strongly supports the relevance of our findings in the H441 cell line and validates the use of H441 cells to model signal transduction in airway epithelial cells.

In skeletal muscle and hepatocytes a role for C/EBPβ and C/EBPδ in glucocorticoid signaling has been implied [23–26]. In these organs, as in the lung, glucocorticoids increase the DNA binding of both C/EBPβ and C/EBPδ. However, this increase in binding is dependent on increased expression of C/EBPβ and C/EBPδ. In contrast to these results, we were not able to detect any increase in protein levels in lung epithelial cells after glucocorticoid treatment, instead experiments using protein synthesis blockers demonstrated the effect to be independent of de novo protein synthesis. As the effect in lung was rapid and not dependent on protein synthesis, this instead suggested that a post-translational mechanism was involved.

Post-translational modifications, such as phosphorylations, regulate the activity of C/EBPs, with regulation of C/EBPβ by phosphorylation being the most well studied [17]. By using an antibody specifically recognizing phosphorylated C/EBPβ we saw that glucocorticoids increased the phosphorylation of one of the two C/EBPβ isoforms at Thr235, suggesting different availability of the two forms to phosphorylation. Since phosphorylation at this site is known to act as a regulatory switch for C/EBPβ activity in p21CIP, IFNγ, and growth hormone signaling [18–20], we suggest that increased phosphorylation at this site could be involved in mediating the glucocorticoid signal. Phosphorylation at this site is known to be mediated by ERK1/2 [18–
but using an antibody that specifically recognizes activated ERK1/2, we saw that ERK1/2 is not activated by glucocorticoids. This was confirmed by blocking MEK1/2, the upstream activator of ERK1/2, which also did not affect the ability of glucocorticoids to increase the binding activity of C/EBPβ. That glucocorticoids affect the phosphorylation status of C/EBPβ at Thr235 by inactivating a phosphatase is unlikely since the use of a broad-spectrum phosphatase inhibitor did not block this effect. Therefore, we suggest that residue Thr235 on C/EBPβ is probably phosphorylated by an uncharacterized kinase not identified in these studies. However, we cannot completely rule out that glucocorticoids act on ERK by a so far unknown mechanism that facilitates the availability of the larger C/EBPβ isoform to ERK. In this case, basal ERK activity would be sufficient and additional activation of ERK would be unnecessary to increase phosphorylation of C/EBPβ at Thr235.

Glucocorticoids are among the most widely prescribed anti-inflammatory drugs, frequently used in treatment of various inflammatory lung disorders, especially asthma. Increasing evidence points towards a central role for the lung epithelium in asthma [27], further highlighting the need to understand glucocorticoid signaling in the lung epithelium. In contrast to asthma, glucocorticoid treatment of the inflammatory lung disorder chronic obstructive pulmonary disease (COPD) is less effective [1,2]. COPD was ranked as the fifth highest cause of death in the world in the year 2002 [28], highlighting the need to find effective treatments for this devastating disease. We have recently demonstrated decreased DNA-binding activity of C/EBPβ in the airway epithelium of smokers with chronic bronchitis and COPD [10]. Considering that C/EBPβ is the most active C/EBP-factor in the human lung epithelium, and that C/EBPβ mediates glucocorticoid-induced gene expression in lung epithelial cells, this suggests that decreased C/EBPβ activity in the lung epithelium of COPD patients could play a part in the decreased glucocorticoid responsiveness that is characteristic for this disease.

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