TGF-β3-null mutation does not abrogate fetal lung maturation in vivo by glucocorticoids

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Shi, Wei, Nora Heisterkamp, John Groffen, Jingsong Zhao, David Warburton, and Vesa Kaartinen. TGF-β3-null mutation does not abrogate fetal lung maturation in vivo by glucocorticoids. Am. J. Physiol. 277 (Lung Cell. Mol. Physiol. 21): L1205–L1213, 1999.—Newborn transforming growth factor (TGF)-β3-null mutant mice exhibit defects of patogenesis and pulmonary development. Glucocorticoids, which play a central role in fetal lung maturation, have been postulated to mediate their stimulatory effects on tropoelastin mRNA expression through TGF-β3 in cultured lung fibroblasts. In the present study, we analyzed the abnormally developed lungs in TGF-β3-null mutant mice and compared the effects of glucocorticoids on gene expression and lung morphology between TGF-β3 knockout and wild-type mice. Lungs of TGF-β3-null mutant mice on embryonic day 18.5 did not form normal saccular structures and had a thick mesenchyme between terminal air spaces. Moreover, the number of surfactant protein C-positive cells was decreased in TGF-β3-null mutant lungs. Interestingly, glucocorticoids were able to promote lung maturation and increased expression of both tropoelastin and fibronectin but decreased the relative number of surfactant protein C-positive cells in fetal lungs of both genotypes. This finding provides direct evidence that glucocorticoid signaling in the lung can use alternative pathways and can exert its effect without the presence of TGF-β3.

Lungs develop from an outpocketing of the ventral foregut endoderm surrounded by splanchnic mesenchyme (6). This process, from airway formation to saccular alveolarization, is regulated by growth factors and cell–cell and cell–extracellular matrix (ECM) interactions (36). Transforming growth factor (TGF)-β isoforms are one key group of growth factors involved in lung organogenesis. The TGF-β1, TGF-β2, and TGF-β3 isoforms all mediate a wide range of biological effects including cell proliferation, differentiation, ECM formation, and cell migration (19). However, the different spatiotemporal expression patterns exhibited by the TGF-β isoforms during embryogenesis and the distinct phenotypes of null mutants demonstrate distinct roles in vivo (9, 11, 20, 22, 26, 31).

Studies on TGF-β isoforms in lung morphogenesis indicated that they have nonredundant roles during developmental processes (12, 15, 37, 42). TGF-β1 is considered to be an inhibitory factor for lung development. In vitro experiments have shown that it inhibits surfactant protein (SP) A and SP-C expression in fetal lungs (15, 37). Exogenous TGF-β1 also inhibits branching morphogenesis in embryonic lung culture (28). Overexpression of constitutively active TGF-β1 in the distal lung epithelium arrests embryonic lung sacculation and epithelial cell differentiation, delaying embryonic lung development (42). However, an abnormal lung phenotype was not observed in TGF-β1-null mutant fetuses, which has been attributed to a maternal rescue effect (12). In contrast to TGF-β1, much less is known about the role of TGF-β3 in lung development. TGF-β3-null mutants show abnormal lung development and defective palatogenesis, leading to death within 24 h after birth (9, 20). The observed abnormal lung morphology in TGF-β3-deficient (TGF-β3−/−) newborn mice includes alveolar hypoplasia, lack of septal formation, mesenchymal thickening, and a decreased number of type II alveolar epithelial cells (9). These findings are reminiscent of immature lungs, which can cause newborn respiratory distress syndrome in human premature infants. A study (35) on the expression of TGF-β3 from primary cultures of fetal lung fibroblasts isolated from different embryonic stages has shown a peak at an early canalicular stage. TGF-β3 is also the most abundant glucocorticoid-induced transcript in fetal rat lung fibroblasts (35). Blocking endogenous TGF-β3 production by antisense oligonucleotides or its activity by neutralizing antibody abrogates the stimulatory effect of glucocorticoids on tropoelastin mRNA expression by cultured fetal lung fibroblasts (40). Because glucocorticoids are known to accelerate pulmonary maturation and are used clinically to prevent respiratory distress syndrome in premature human infants, it is important to understand the molecular and cellular mechanisms of action of glucocorticoids in lung development and their connection to TGF-β3 in vivo. This is the focus of the present study.

MATERIALS AND METHODS

Homozygous TGF-β3 Knockout Mice

Heterozygous TGF-β3(+/−) mice were previously generated in our laboratory (9). Homozygous TGF-β3(−/−) fetal mice were produced by timed mating between TGF-β3 heterozygous male and female mice. The finding of a vaginal plug was counted as embryonic day (ED) 0. The pregnant mice were killed on ED18.5, and the fetuses were removed by
cesarean section. The fetal lung was excised en bloc and dissected into each individual lobe under a dissecting microscope. Different lobes were processed for either fixation or RNA extraction. Genomic DNA was isolated from a part of the tail, and the genotype was confirmed by Southern blot analysis (9).

Preparation of Tissue Sections

The superior lobe of the right lung was fixed for 4 h in 4% paraformaldehyde-PBS at 4°C. The fixed tissues were then dehydrated in ethanol and embedded in paraffin. Coronal sections were cut at 6 μm thickness and mounted on Superfrost glass slides (Fisher Scientific, Pittsburgh, PA). The slides were dried at 37°C overnight and 60°C for 1 h. The sections across the middle part of the lobe were chosen for this study. Hematoxylin and eosin staining was used to study the histological structure of different sections. Comparison between wild-type and TGF-β3(−/−) specimens for either gene expression or morphology was performed among littermates.

Morphometry

The area of terminal respiratory air spaces was measured with SigmaScan version 3.1 (Jandel Scientific, Corte Madera, CA). Multiple measurements were performed on randomly selected 0.04-mm² fields located at the distal part of the fetal lungs. The proportion of lung comprising terminal air spaces is presented as a percentage of the total area of the lung section. The mean ± SD was obtained from sections of three individual fetal lungs of wild-type or TGF-β3 knockout genotype.

Immunohistochemistry

Lung sections were first deparaffinized in xylene, followed by rehydration in series of different concentrations of ethanol. The endogenous hydrogen peroxidase was quenched with 3% H₂O₂ in methanol for 10 min. Zymed Histostain-Plus (Zymed, South San Francisco, CA) was used for the following staining procedures.

SP-C immunohistochemical staining. Rabbit anti-proSP-C polyclonal antibody was a generous gift from Dr. Jeffrey Whitsett (Children’s Hospital Research Foundation, Cincinnati, OH) (34). The sections were incubated with antibody diluted 1:1,000 in PBS-0.2% Triton X-100 overnight at 4°C. Positively stained epithelial cells were counted, and a comparison between wild-type control and null mutant mice was performed.

Anti-fibronectin immunostaining. A rabbit anti-fibronectin polyclonal antibody was purchased from Sigma (St. Louis, MO). The sections were incubated with antibody diluted 1:1,000 for 1 h at room temperature.

Elastin staining. Hart’s elastin staining method was used (14). Briefly, the deparaffinized section was first treated in potassium permanganate for 30 min followed by a rinse in a 5% oxalic acid solution and then stained in resorcin-fuchsin solution overnight and finally in van Gieson’s solution for 1 min. The number of positively stained fibers and their intensity were compared between wild-type control and null mutant mice.

Total RNA Isolation and RT

The lungs were first dissected into each individual lobe. To eliminate the contribution of large blood vessels and large bronchi, approximately one-third of the peripheral portion of the lung was dissected and immediately frozen in liquid nitrogen. The tissues were further processed for total RNA isolation with a Qiagen RNeasy kit (Qiagen, Santa Clarita, CA). The quality of isolated RNA was checked by agarose gel electrophoresis before the RT reaction.

About 200 ng of total RNA in 25 μl containing 1.25 μg of oligo(dT) (Pharmacia), 50 mM Tris·HCl, pH 8.0, 70 mM KCl, 10 mM MgCl₂, 1 mM deoxynucleotide triphosphates, 10 mM dithiothreitol, 40 U of RNasin ribonuclease inhibitor (Promega, Madison, WI), and 1,000 U of Moloney murine leukemia virus reverse transcriptase (Life Technologies, Gaithersburg, MD) were used for each RT reaction at 37°C for 90 min. The product of the RT was diluted fivefold and applied to competitive PCR.

Primers and Competitive RT-PCR

The competitive RT-PCR method has been previously described (41). For tropoelastin competitive PCR, a 291-bp fragment of mouse tropoelastin cDNA was amplified by PCR with the primers 5‘-TGCCAAAGCTGCTGCTAAGGCT-3’ and 5‘-AGTCCAAAGCCAGTCTTGTGCTG-3’. In addition, a 344-bp competitor template fragment was constructed from v-erbB DNA by PCR with a pair of composite primers, which were attached with the same nucleotide sequences of tropoelastin primers at the ends. In this way, the tropoelastin-specific primer sequences were incorporated into both ends of the competitive cDNA template, which can also be amplified with the same pair of tropoelastin primers.

Primers and competitor templates for β-actin, fibronectin, and SP-C genes were designed in a similar way as previously described (39). Competitive RT-PCRs were performed on cDNA samples described above with known amounts of competitive templates. The reaction mixture contained 10 mM Tris·HCl (pH 9.4), 50 mM KCl, 2 mM MgCl₂, 0.01% gelatin, 0.1% Triton X-100, 20 pmol of the primer sets, 100 μM deoxynucleotide triphosphates, and 0.5 U of Advantage KlenTaq DNA polymerase (Clontech, Palo Alto, CA) in a total volume of 50 μl. PCR amplification was carried out in a Robocycler (Stratagene, La Jolla, CA) with a modification of a previously described assay (41) for the TGF-β3 type II receptor (35 cycles of denaturation at 94°C for 2 min, annealing at 62°C for 2 min, and extension at 72°C for 2 min).

For quantitation, a standard curve was always made with a series of cDNA standard dilutions as samples (Fig. 1, A and B). The concentration of cDNA was determined spectrophotometrically. As an internal control, β-actin competitive PCR was performed on the same samples. As a negative control for mouse genomic DNA or v-erbB DNA, un-reverse-transcribed total RNA and adenoviral DNA were also included in the competitive PCR assays.

Gel Electrophoresis and Quantitation of PCR Products

A 3% agarose gel (3:1 mixture of NuSieve and SeaKem, FMC BioProducts, Rockland, ME) was used to separate target and competitor PCR products. The intensity of each band was determined by densitometric analysis with ImageQuant band-analyzing software (Molecular Dynamics, Sunnyvale, CA). RNA samples from three individual wild-type or TGF-β3 knockout fetuses were analyzed, and triplicate competitive RT-PCRs were performed on each sample. All data are reported as means ± SD. Differences between means were statistically tested by a Wilcoxon rank sum test (10). P values < 0.05 were considered to be significant.

Maternal Dexamethasone Treatment of Mice

Pregnant mice were injected intramuscularly with dexamethasone or saline consecutively on ED15.5 and ED16.5.
The dosage of water-soluble dexamethasone (Sigma) was 2 µg/g body wt.

RESULTS

Morphological Characterization of TGF-β3 Knockout Fetal Lungs on ED18.5

The size and lobation of the ED18.5 fetal lungs from TGF-β3 knockout mice were comparable to those of wild-type mice. Microscopic features of wild-type and null mutant fetal lungs on ED18.5 are shown in Fig. 2. In wild-type mice of this age, saccule formation can be seen in the periphery of the lung, respiratory tubules were dilated, and the cellular, thick mesenchyme between the terminal air spaces became thinner, with many septum-forming buds extended into the dilated lumen compared with those in the fetal lungs at an earlier stage. These appearances are characteristic of saccular morphology (Fig. 2, A and B), which provides the structural basis for alveolarization after birth. In comparison, the saccule formation of ED18.5 fetal lungs from TGF-β3 knockout mice was retarded, and the thickness of the mesenchyme between the terminal air spaces was increased. The area of the terminal air spaces in the total lung section was reduced to 33.6 ± 4% in TGF-β3 knockout fetal lung compared with 50 ± 6% in wild-type fetal lungs (P < 0.005). The density of cells in the mesenchymal region of TGF-β3 knockout fetal lungs was relatively high compared with that in wild-type fetal lungs (Fig. 2, E and F). The number of saccular structures in TGF-β3 knockout fetal lungs was smaller than that in wild-type fetal lungs and was restricted to a narrow region directly underneath the pleural membrane. Moreover, TGF-β3-null mutants displayed abnormally dilated intrapulmonary blood vessels (Fig. 2E) as previously reported (9).

Because TGF-β3 is a possible candidate for the mediating effects of glucocorticoids in fetal lungs, lack of TGF-β3 function was predicted to cause insensitivity to glucocorticoid treatment. To test this hypothesis, we compared the morphology of ED18.5 fetal lungs with and without maternal administration of dexamethasone on ED15.5 and ED16.5. Dexamethasone treatment enhanced saccular formation in TGF-β3 knockout fetal lungs (Fig. 2, G and H) as well as in wild-type fetal lungs (Fig. 2, C and D). The proportion of terminal air space area was increased in both genotypes after dexamethasone treatment (65 ± 8% in wild-type fetal lungs, P < 0.025; 54 ± 6% in TGF-β3 knockout fetal lungs, P < 0.005). However, the effect was greater in wild-type fetal lungs, which exhibited morphology approaching the postnatal alveolar stage.

Decreased Expression of Tropoelastin and Fibronectin in TGF-β3-Null Fetal Lungs

TGF-β isoforms are well-known stimulators of the expression of genes encoding several ECM proteins...
Moreover, TGF-β3 has been shown to induce tropoelastin gene expression in primary lung fibroblasts isolated from fetal rats. Also, inactivation of TGF-β3 function in cultured lung fibroblast cells has been reported to block the stimulatory effect of dexamethasone on tropoelastin expression. Therefore, we examined the production of the ECM proteins tropoelastin and fibronectin in fetal lungs of TGF-β3 knockout mice with and without dexamethasone treatment.

Tropoelastin mRNA expression in TGF-β3 knockout fetal lungs was ~70% of the expression level in wild-type control lungs (P < 0.05; Fig. 1, C and D). However, maternal administration of dexamethasone resulted not only in a 300% increase in tropoelastin expression compared with untreated TGF-β3 knockout fetal lungs (P < 0.05) but also in a 230% increase in tropoelastin expression compared with untreated wild-type fetal lungs (P < 0.05). This result was confirmed by Northern blot analysis (data not shown). Therefore, TGF-β3 is not absolutely required for dexamethasone-induced tropoelastin expression. Because tropoelastin is the monomeric form of elastin that constitutes elastin fibers, elastin fibers of ECM components were visualized by elastin-specific staining (Fig. 3). In wild-type fetal lungs on ED18.5, elastin fibers appeared thicker, and bundles of elastin fibers were easily seen in areas of septal formation. Compared with wild-type fetal lungs, the amount of elastin fibers in TGF-β3 knockout fetal lungs was decreased. Also, the fibers were thinner and appeared in fewer mature saccules. In contrast, thick...
bundles of elastin fibers were seen in dexamethasone-treated TGF-β3 knockout fetal lungs.

Besides tropoelastin, expression of fibronectin was slightly reduced in TGF-β3 knockout fetal lungs compared with wild-type control lungs (Fig. 4). Treatment with dexamethasone resulted in a 60–80% increase in fibronectin expression in both genotypes. Immunostaining of fetal lung sections for fibronectin did not show any striking differences in the distribution pattern of fibronectin between wild-type and TGF-β3 knockout samples (data not shown).

**Type II Epithelial Cells and SP-C Expression**

Differentiation of epithelial cells is characteristic of fetal lungs at the saccular stage. Type II epithelial cells synthesize and secrete SPs such as SP-C. Using a quantitative competitive RT-PCR method, we found that SP-C expression in TGF-β3-null fetal lungs was decreased by 50% compared with that in wild-type fetal lungs (Fig. 5). The relative number of SP-C-positive epithelial cells shown by proSP-C immunohistochemical staining was also decreased by 30% (Fig. 6, A and B). ProSP-C immunoserum also detects immature precursor cells, which explains the positive staining of some cells that do not appear to be typical type II epithelial cells. Interestingly, maternal treatment with dexamethasone decreased the mRNA levels of SP-C expression in total lung tissues (Fig. 6, C and D) and the relative number of SP-C-positive epithelial cells in the fetal lungs of both wild-type and TGF-β3 knockout mice by 60% (Fig. 6, E and F).

**DISCUSSION**

**Role of TGF-β3 in Fetal Pulmonary Development**

Lung development is a complicated biological process. Morphologically, it can be divided into four stages (32). Initially, the primitive lung bud develops into bronchi under the induction of the mesenchyme surrounding the distal ends of the endoderm-derived epi-
thelial tubules. This pseudoglandular stage in mice spans ED9.5 to ED16.5. During the following canalicu-
lar stage (approximately ED16.5 to ED17.5 in mice), centrifugal branching morphogenesis is continued to
give rise to the terminal respiratory epithelial cells and pulmonary vascularization is initiated, which forms close contacts with respiratory airways. The terminal tubules are then expanded into terminal sacs with
differentiated type I and type II epithelial cells. The terminal sac stage in mice occurs from ED17.5 to
postnatal day 5. The final alveogenesis stage starts
around postnatal day 5 in mice with completion of
alveolar septal formation, generating mature respira-
tory units with gas-exchange functions. With the use of
transgenic and gene-targeting methods, a group of
growth factors including bone morphogenetic protein-4,
TGF-β1, epidermal growth factor, keratinocyte growth factor, fibroblast growth factor, and platelet-derived growth factor has been implicated in lung development (2, 3, 13, 17, 29, 30). The previous study by Kaartinen et al. (9) on TGF-β3 knockout mice suggested that lack of TGF-β3 function causes an immature lung phenotype in newborn mice (9). Consistent with their previous observations, the size and lobation of the lungs in TGF-β3 knockout mice were not found to be obviously abnormal, which indicates that branching morphogenesis in the pseudoglandular and canalicular stages are not dramatically disturbed by a lack of TGF-β3. However, the morphological abnormalities in TGF-β3 knockout mice are obvious in late maturation stages in fetuses. Because the deft palate in TGF-β3-null mice may indirectly change lung morphology in newborn pups suffering respiratory distress or aspiration after birth, our present study focused on embryonic fetal lungs on ED18.5 (birth occurs around ED19).

The abnormal morphology of TGF-β3 knockout fetal lungs on ED18.5 was not a simple arrest of pulmonary development at a particular stage. Parts of nearly mature saccular structures can still be seen in TGF-β3 knockout lungs, but the generally increased thickness of the mesenchyme between the terminal air spaces with more layers of cells indicates an abnormal phenotype. Normally during the late prenatal period, alveolar air space area gradually increases and mesenchymal thickness decreases. Treatment with dexamethasone in the late pseudoglandular and early canalicular stages promoted saccular structure formation in both wild-type and TGF-β3 knockout mice. However, the morphological maturation of knockout fetal lungs still appeared to be retarded in comparison with that of wild-type fetal lungs after maternal dexamethasone treatment. This indicates that dexamethasone cannot fully restore the phenotype caused by TGF-β3 deficiency, which is consistent with the observation that morphometric changes in the lung parenchyma after glucocorticoid exposure are not analogous to normal maturation changes (38).

The mechanism of mesenchymal thinning is poorly understood. In wild-type late-gestation lungs, the number of apoptotic cells is very low, and, therefore, it is very unlikely that mesenchymal thinning results from programmed cell death (3, 19). The hypercellular lung phenotype, with a thick mesenchyme and poorly developed prealveolar spaces, has also been described in corticotropin-releasing hormone (CRH)-deficient mice (18). The lethal phenotype of these mice can be rescued by prenatal administration of glucocorticoids; this provides further evidence for the importance of glucocorticoid signaling in late-term pulmonary maturation (18, 19). In wild-type mice, mesenchymal cell proliferation ceases during the late-gestational period, whereas in CRH-deficient mice, cell proliferation continues (19). The striking similarity between the pulmonary phenotypes of the CRH-deficient and TGF-β3-deficient mice suggests that the impaired lung maturation in TGF-β3-null mutant mice is caused by a defect in glucocorticoid signaling that leads to the continued proliferation of mesenchymal cells. The fact that the TGF-β3-null mutant phenotype can, at least partially, be restored by exogenous administration of glucocorticoids would indicate that glucocorticoid-induced lung maturation in TGF-β3-null mutant mice works above certain thresholds; i.e., in these mice, the endogenous glucocorticoid level is not sufficient to reduce mesenchymal cell proliferation.

TGF-β3 Regulates Expression of Tropoelastin and Fibronectin in Fetal Lungs

Elastin is one critical component of lung ECM by providing structural recoil properties essential for respiratory function. The expression of tropoelastin, the monomeric form of elastin, begins in the pseudoglandular stage, increasing during fetal lung development and reaching a peak in the alveolar stage (16). Both TGF-β3 and tropoelastin transcripts are induced by dexamethasone treatment (40). Blockade of endogenous TGF-β3 production with antisense oligonucleotides or of TGF-β3 activity with neutralizing antibodies has been reported to abrogate glucocorticoid-stimulated tropoelastin expression in cultured rat lung fibroblasts, suggesting that glucocorticoid-induced tropoelastin expression is mediated via TGF-β3 (40). Using TGF-β3-null mutant mice, we were able to further test the latter hypothesis in vivo. Tropoelastin expression in ED18.5 fetal lungs of TGF-β3-null mice was slightly but significantly reduced compared with that in wild-type control lungs. Maternal dexamethasone treatment at the late pseudoglandular and early canalicular stages greatly augmented tropoelastin expression not only in wild-type fetal lungs (3-fold) but also in TGF-β3 knockout fetal lungs (2.3-fold). The increase in tropoelastin expression regardless of TGF-β3 genotype after maternal dexamethasone treatment indicates that TGF-β3 is not the only factor mediating the effect of dexamethasone in vivo, and other molecules and pathways may compensate for the absence of TGF-β3.

In addition to tropoelastin, another important ECM component, fibronectin, was found to be expressed at a somewhat lower level in TGF-β3 knockout fetal lungs. Fibronectin is an adhesive substrate in the ECM that can bind to integrin receptors on cell surfaces to affect cell proliferation, differentiation, migration, and apoptosis. The expression of fibronectin in murine embryonic lungs has been detected in the mesenchyme and parabronchial cells at the pseudoglandular stage (23). Intense staining of fibronectin was observed at the branching points of developing airways in wild-type fetal lungs. Blockade of fibronectin binding to its integrin receptors in cultured murine lung explants inhibited branching morphogenesis (24, 25). In the late stages of pulmonary development, fibronectin expression is normally decreased; however, it has been shown that fibronectin promotes fetal lung vasculogenesis and angiogenesis in vitro (7, 8). Whether the reduced fibronectin expression is related to the observed phenotypes in TGF-β3-null mutant mice requires further study.
TGF-β3 Regulates Epithelial Cell Differentiation

The significant reduction in SP-C mRNA expression and the relative number of SP-C-positive epithelial cells in TGF-β3 knockout fetal lungs further suggests, but does not directly demonstrate, a concomitant decrease in type II epithelial cells and/or of their function. The relative decrease in the number of type II cells staining with SP-C antibody indicates that TGF-β3 is required for normal type II epithelial cell differentiation. Surprisingly, in our experiments, maternal dexamethasone treatment reduced both SP-C expression and the number of SP-C-positive epithelial cells. In rats, maternal dexamethasone (1 mg/kg) treatment at the late-embryonic stage increased SP-C mRNA levels in fetal lungs (27). Induction (10- to 30-fold) of SP-C expression was also observed in human fetal lungs cultured as explants in the presence of dexamethasone (100 nM) (1, 33). However, maternal betamethasone treatment at a late-gestation stage of rabbits reduced SP-C expression > 50% in fetal lungs (4). Interestingly, Grummer and Zachman (5) reported that dexamethasone (10⁻¹⁰ to 10⁻⁸ M) in murine type II epithelial cells initially increased SP-C expression but that this increase was followed by a constant reduction in SP-C expression, reaching the lowest level after 48 h. Like retinoic acid, which has a more variable (either positive or negative) effect on SP-C expression than on SP-A and SP-B expression, the concentration and length of dexamethasone treatment, the species, and the embryonic stage could account for the variable responses in SP-C expression. Another possibility is that dexamethasone can accelerate the differentiation of SP-C-positive precursor epithelial cells. However, SP-C expression in individual type II cells did not appear to change because the intensity of SP-C staining was comparable.

In summary, our in vivo experimental data from the TGF-β3 knockout mouse model indicates that TGF-β3 is essential for the morphological and functional maturation of the fetal lung and the normal expression levels of tropoelastin, fibronectin, and SP-C during lung development. In concordance with the studies of others (19), exogenous glucocorticoids enhance the fetal lung maturation and expression of tropoelastin in wild-type mice. Moreover, the opposite response of tropoelastin and SP-C expression to glucocorticoid treatment suggests a dissociation between the processes involved in connective tissue remodeling and epithelial cell differentiation. Our present results demonstrate similar, albeit less pronounced, changes in TGF-β3-null mutant mice and would argue that glucocorticoid signaling in the lung does not absolutely require TGF-β3 in vivo.

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