FAM13A as potential therapeutic target in modulating TGF-beta-induced airway tissue remodeling in COPD

Anthony Tam¹,³, Pascal Leclair¹, Ling Vicky Li², Chen X Yang³, Xuan Li³, Dominik Witzigmann⁴,⁵, Jayesh A Kulkarni⁴,⁵, Tillie-Louise Hackett³, Delbert R Dorscheid³, Gurpreet K Singhera³, James C Hogg³, Pieter R Cullis⁴,⁵, Don D Sin³*, Chinten James Lim¹**.

¹ Department of Pediatrics, University of British Columbia, Vancouver, BC, Canada
² Department of Pathology, University of British Columbia, Vancouver, BC, Canada
³ University of British Columbia (UBC) Center for Heart Lung Innovation, St. Paul’s Hospital, Vancouver, British Columbia, Canada
⁴ University of British Columbia (UBC), Department of Biochemistry and Molecular Biology, Vancouver, British Columbia, Canada
⁵ NanoMedicines Innovation Network, Vancouver, BC, Canada

*Co-corresponding authors
† Author for correspondence

Chinten James Lim, PhD
3092-950 West 28th Avenue, Vancouver, B.C. Canada V5Z-4H4
Tel: 604 8752000 ext 4795
E-mail: cjlim@mail.ubc.ca

Running head: FAM13A and airway epithelial remodeling
Abstract

Genome-wide association studies have shown that a gene variant in the Family with Sequence Similarity 13, Member A (FAM13A) is strongly associated with reduced lung function and the appearance of respiratory symptoms in patients with Chronic Obstructive Pulmonary Disease (COPD). A key player in smoking-induced tissue injury and airway remodeling is the transforming growth factor β1 (TGFβ1). To determine the role of FAM13A in TGFβ1 signaling, FAM13A-/- airway epithelial cells were generated using CRISPR-Cas9, while over-expression of FAM13A was achieved using lipid nanoparticles. Wildtype (WT) and FAM13A-/- cells were treated with TGFβ1, followed by gene and/or protein expression analyses. FAM13A-/- cells augmented TGFβ1-induced increase in COL1A1 and MMP2 expression compared to WT cells. This effect was mediated by an increase in CTNNB1 expression in FAM13A-/- cells compared to WT cells after TGFβ1 treatment. FAM13A over-expression was partially protective from TGFβ1-induced COL1A1 expression. Finally, we showed that airway epithelial-specific FAM13A protein expression is significantly increased in patients with severe COPD compared to control non-smokers, and negatively correlated with lung function. In contrast, β-catenin (CTNNB1), which has previously been linked to be regulated by FAM13A, is decreased in the airway epithelium of smokers with COPD compared to non-COPD subjects. Together, our data showed that FAM13A may be protective from TGFβ1-induced fibrotic response in the airway epithelium via sequestering CTNNB1 from its regulation on downstream targets. Therapeutic increase in FAM13A expression in the airway epithelium of smokers at risk for COPD, and those with mild COPD, may reduce the extent of airway tissue remodeling.

Key words: FAM13A, airway epithelium, TGFβ1, COPD, lipid nanoparticles
Introduction

Previous reports have shown that Chronic Obstructive Pulmonary Disease (COPD) is associated with a decline in lung function with symptoms of chronic cough, sputum production and breathlessness on exertion in approximately 20% of adults over 40 years of age (2, 9, 40). Recently, genome wide association studies (GWAS) and large-scale meta-analyses have shown that several genes are reproducibly associated with lung function in patients with COPD (11, 36, 37, 41, 43, 47). One of these is the Family with Sequence Similarity 13, Member A (FAM13A), which has been strongly related to reduced lung function and with increased risk of COPD (10, 20, 44). In response to repetitive cigarette smoke, it has been shown in COPD that the airway epithelium releases transforming growth factor-β1 (TGFβ1) and is thought to be associated with airway fibrosis and luminal narrowing (18, 23, 42, 49). Remodelled airways in COPD demonstrate changes in markers of epithelial-mesenchymal transition (EMT), including increased collagen type 1 (COL1A1), matrix metalloproteinase 2 (MMP2) and vimentin (VIM), and decreased E-cadherin (CDH1), which may be exacerbated by cigarette smoke-induced oxidative stress, leading to over-activity of TGFβ (4, 39).

The mechanisms connecting the role of FAM13A and airway tissue remodeling are not well understood. Jiang and colleagues have shown that FAM13A interacts with protein phosphatase 2A and glycogen synthase kinase 3β for the ubiquitination and degradation of β-catenin (CTNNB1) (27). TGFβ1-induced matrix synthesis in the airway epithelium has been shown to reduce the levels of E-cadherin, thereby liberating CTNNB1 from its complex into the cytoplasm (21). Free and unbound CTNNB1, which has not been subjected to proteasomal degradation, can translocate into the nucleus and serve as transcriptional co-activators to its DNA-binding partner, TCF/Lef, to initiate the transcription of CTNNB1-regulated genes (25, 52, 53, 59). However, the mechanistic role of FAM13A in modulating the expression of CTNNB1 in TGFβ1-induced EMT responses is not well characterized.

In this study, we used CRISPR-Cas9 gene editing technology in airway epithelial cells to determine the effects of FAM13A on TGFβ1-mediated response, and characterize FAM13A protein expression in human lung tissues from individuals with and without COPD. First, we showed that loss of FAM13A increased TGFβ1-induced rise in COL1A1, MMP2 and CTNNB1 expression in human airway epithelial cells. Disruption of CTNNB1 using gene silencing technology and pharmacologic inhibitors consistently decreased TGFβ1-induced rise in COL1A1 gene expression. Over-expression of FAM13A using lipid nanoparticles decreased TGFβ1-induced rise in COL1A1 protein expression. Finally, we demonstrated that FAM13A expression is up-regulated, whereas CTNNB1 expression is down-regulated in the airway epithelium of patients with COPD compared to subjects without COPD. Collectively, these data indicate that FAM13A may have a protective role against epithelial remodeling that leads to progressive narrowing of the airways in COPD.
Materials and Methods

Human lung tissues for immunohistochemistry

Formalin-fixed paraffin-embedded (FFPE) human lung tissue samples were obtained from five non-smoking controls, nine smokers with COPD, with Global initiative for chronic obstructive Lung Disease (GOLD) stage 2 (moderate) disease and eight smokers with GOLD3 or 4 (severe or very severe). All of these samples were collected following informed consent from patients undergoing thoracic surgery who donated lung tissues to the UBC James Hogg lung tissue registry located at St. Paul’s Hospital approved by the Providence Health Care Research Ethics Board (PHCREB) H00-50110. All experiments on human samples were conducted in accordance with existing guidelines under the PHCREB protocol approved by the University of British Columbia (B17-0027). The previously reported patient demographics are summarized in Table 1 (50).

Immunohistochemistry

FFPE sections of human lung tissues were stained with antibodies against FAM13A (Sigma HPA038109) using the Bond Polymer Refine Red Detection kit on the Leica Bond Autostainer according to the manufacturer’s protocol. Slides were cover-slipped and scanned using the Aperio imaging system (Leica Biosystem; Concord, Ontario).

Human airway epithelial gene expression cohort 1

To provide gene expression evidence of airway epithelial transition in smokers with or without COPD, human small airway epithelial (10th-12th generation bronchi) gene expression data were collected from sixteen Gene Expression Omnibus (GEO) microarray studies (GSE19667, GSE5058, GSE11784, GSE11906, GSE8545, GSE10006, GSE20257, GSE11952, GSE63127, GSE13933, GSE4498, GSE18385, GSE17905, GSE13931, GSE19407, GSE7832). All of these studies were pooled into one cohort as these studies were generated using the same microarray platform (U133A Plus 2.0 array) and reported by the same lab. Raw data (.CEL files) downloaded from the GEO were tested for batch effect and outliers using principal component analysis. Since no obvious batch effect was present, the combined data were normalized using the Robust Multi-array Average (RMA) method. After confirmation and removal of three outliers, this cohort contains 68 non-smokers, 101 smokers and 42 smokers with COPD. A summary of the patient demographics is presented in Table 2.

Human airway epithelial gene expression cohort 2

To determine validate the gene expression directionality of CTNNB1 in COPD, we used gene expression data of airway epithelia obtained from individuals with and without COPD at the British Columbia Cancer Agency (BCCA) (GSE37147). In brief, RNA was obtained from small airways of smokers (with or without COPD) through a research bronchoscopy. These samples were then processed and hybridized to Affymetrix Human Gene 1.0 ST Arrays. A total of 269 arrays from 267 subjects were hybridized. Data from the 269 microarrays were used for RMA normalization. Data from 238 subjects were used in the downstream analysis to determine the relationship of gene expression with COPD-related phenotypes. Raw gene expression from GSE37147 (.CEL files) were downloaded from GEO, and then normalized using the Robust Multichip Average (RMA) algorithm. The association of CTNNB1 gene expression and FEV1 % predicted, FEV1/FVC, COPD, smoking status were tested in linear regressions adjusted for age, sex and smoking status. The analyses were done in R (3.6.0). The patient demographics were
previously reported (48) and presented below in Table 3.

**Cell culture and transfections**

The human airway epithelial cell line, 1HAEo, was obtained from Dr. Dieter Gruenert (UCSF) (19) and cultured in DMEM (Invitrogen) with 10% fetal bovine serum-FBS (ThermoFisher 12483020) and 1% penicillin-streptomycin (ThermoFisher 15140122). For all procedures, the media and applicable supplements were replaced every 2 days.

For gene knockout with CRISPR-Cas9, 5'-AGCAGGATGAAGTTCGACAT-3’ (IDT), which corresponded to a unique guide RNA targeting an early canonical gene exon of human FAM13A, was cloned into BbsI digested pX458 (Addgene 48138) to yield pX458-hFAM13A-4.2. Following sequence confirmation, this clone was propagated in DH5α E. coli (13). Guide sequences were chosen based on high-quality scores, and minimal off-target sites and mismatches according to crispr.mit.edu. DNA plasmids were transfected into 1HAEo cells using Lipofectamine 3000 (Invitrogen) for 24h according to the manufacturer’s protocol. Cells positive for Cas9-GFP expression were single cell-sorted by flow cytometry into 96-well plates and maintained in complete media until colonies were formed. The expanded clones were secondarily screened for FAM13A knockdown using Western blots, and candidate null clones were confirmed by PCR sequencing of the targeted genomic loci covering the PAM motif. For experiments, wildtype (WT) and FAM13A−/− cells were cultured in reduced serum (1% FBS) and treated with 10ng/mL TGFβ1 for 7 days (26, 46).

For gene silencing with RNAi using lipofectamine 3000 (L3000015; ThermoFisher), cells were cultured in 1% FBS and pre-treated with 25nM silencer-select scrambled or CTNNB1 siRNA (ThermoFisher 4390843 or s19753) for 2 days, followed by another round of siRNA treatment in the presence or absence of 10ng/mL TGFβ1 (BioLegend 580704) for 5 days.

For the intervention study, 1HAEo cells were cultured in 1% FBS and pre-treated with 100ng/ml PKF118-310 (inhibits TCF4/CTNNB1 complex) (K4394; Sigma) for 2 days, followed by PKF118-310 in the presence or absence of 10ng/mL TGFβ1 (BioLegend 580704) for 5 days.

**Real-time PCR**

PCR methods and analysis have been previously described (50). In brief, human TaqMan probes (Life Technologies) were used to measure expression of FAM13A (Hs00208453_m1), COL1A1 (Hs00164004_m1), MMP2 (Hs01548727_m1) and CDH1 (Hs01023894_m1) and CTNNB1 (Hs99999168_m1) with normalization to GAPDH (Hs02758991_g1). Gene expression was expressed as Δct (relative quantitation) and ΔΔct (fold changes) with normalization to control values.

**Western blot**

Western blot methods and analysis have been previously described (50, 51). In brief, 25µg of whole cell lysate extracted using cell lysis buffer (Cell Signaling 9803) or nuclear/cytoplasmic lysate extracted using the NE-PER Nuclear and Cytoplasmic Extraction Reagents (ThermoFisher 78833) was resolved by 10% SDS-PAGE, and transferred to nitrocellulose (Biorad 1704159) using the Trans-Blot Turbo Transfer System (Biorad 17001917). Membranes were incubated with primary antibodies against FAM13A (Sigma HPA038109), COL1A1 (Abcam Ab34710), total
CTNNB1 (Cell Signaling 9562S), active CTNNB1 (Cell Signaling 8814S), MMP2 (Cell Signaling 40994), CDH1 (Abcam ab40772), β-actin (Sigma A1978) and HDAC2 (Cell Signaling 5113S), followed by incubation with IRDye anti-rabbit and/or anti-mouse IgG secondary antibodies (Lior 926-68071 and 926-32210) and visualized using Odyssey CLx (LI-COR Biosciences).

**Immunofluorescence staining and confocal microscopy**

To visualize COL1A1 and CTNNB1 protein expression, cells were fixed with formalin, permeabilized by Triton X and incubated with primary antibodies against COL1A1 (Cell Signaling 39952) and total CTNNB1 (Cell Signaling 9562S) overnight at 4°C, and stained with Alexa Fluor® 488 goat anti-mouse IgG and 594 goat anti-rabbit IgG (Life Technologies) for 2h at room temperature. To visualize the effect of TGFβ1 on filamentous actin (F-actin) organization, WT and FAM13A−/− cells were fixed and stained with fluorescein-phalloidin (ThermoFisher F432) as per the manufacturer’s instruction. Slides were counterstained with DAPI for cell nuclei (Sigma 10236276001), cover-slipped and visualized using confocal microscopy at 20X magnification. Fluorescence intensity from the entire image was normalized to nuclei count using Image J.

**Preparation of lipid nanoparticles**

Lipid nanoparticles (LNP) composed of ionizable lipid/phosphatidylcholine/cholesterol/PEG-lipid (50/10/38.5/1.5mol %) were prepared using rapid-mixing techniques (32, 35)(1). Lipid components dissolved in ethanol (10 mM total lipid) were combined with 25 mM sodium acetate pH 4 and dialyzed into the same buffer to remove solvent. The resulting pre-formed vesicles were concentrated and stored until use in transfections. Using a benchtop-mixing procedure described elsewhere (33), we combined mRNA with LNP at a ratio of 29 μg RNA per μmol lipid immediately before dilution into cell media.

**Lipofectamine and LNP-mediated delivery of GFP and FAM13A mRNA**

First, LNP-mRNA uptake efficiency was evaluated by incorporation of the non-exchangeable lipid dye DiD (1,1-dioctadecyl-3,3,3,3-tetramethylindodicarbocyanine) at 0.2 mol%. Second, to validate the transfection efficiency of the developed LNP system, CleanCap eGFP mRNA (L-7201; Trilink Biotech) was encapsulated and used for transfection of 1HAEo cells at a final concentration of 3 μg mRNA/mL for 24h. Lipofectamine 3000 was used as a positive control for the transfection of eGFP mRNA. To over-express FAM13A, the coding sequence of the human FAM13A mRNA, transcript variant 2 (NM_001015045.2) was obtained from Trilink BioTechnologies (L-7007). FAM13A mRNA was encapsulated into LNPs as described above. Cells were treated with TGFβ1 for 3 days, followed by a refreshment of TGFβ1 + LNPs entrapping FAM13A mRNA at 1 or 3 μg mRNA/ml for 4 days. To model a more acute induction of total and active CTNNB1 in both cytoplasmic and nuclear fractions, cells were treated with TGFβ1 for 1 day, followed by a refreshment of TGFβ1 + LNPs entrapping FAM13A mRNA at 3 μg mRNA/ml for 3 days. RNA and protein were extracted for real time PCR and Western blot analyses, respectively.

**Flow Cytometry Analysis**

To assess the percentages of GFP+ cells, 1HAEo cells were analyzed by flow cytometry (Gallios, Beckman Coulter). Briefly, cells were lifted with trypsin (Invitrogen 25200056) for 2 min at 37 °C followed by neutralization with 10% FBS DMEM media, fixed with 10% formalin for 10
minutes, and resuspended in 2% FBS PBS. Kaluza for Gallios acquisition software (Beckman
Coulter) was used to analyze the % GFP+ and DiD+ cells in the samples.

Cell imaging
To observe single cell dynamics in real time, cells were seeded at low density in 10% FBS DMEM
and allowed to attach for 5h. Fresh complete media was replaced prior to live cell imaging every
min for 3h. To observe changes in cellular morphology relative to density over time, WT and
FAM13A−/− 1HAEo cells were seeded at low density in 10% FBS DMEM with fresh complete
media containing TGFβ1 replaced every 2 days.

Statistical methods
In-vitro and ex-vivo study data were tested for normality prior to the selection of a parametric
(normal distribution) or Mann Whitney (non-normal distribution) t-test, Kruskal Wallis multiple
comparisons test, one-way ANOVA with Bonferroni’s multiple comparisons test, and linear
regression test, where appropriate. All data were analyzed using GraphPad Prism 8 (GraphPad
Software Inc) and were expressed as mean ± SEM. Statistical significance was considered at P <
0.05.
Results

Expression of FAM13A is increased and CTNNB1 is decreased in the airway epithelium of patients with COPD.

To determine the relationship between FAM13A and CTNNB1 expression in airway epithelium, FAM13A expression in lung tissue samples from non-smoking controls and subjects with COPD was evaluated by immunohistochemistry. The demographics of this cohort is shown in Table 1. Representative images of airway epithelial-specific FAM13A protein expression in non-smoking controls and COPD patients with GOLD 2 and GOLD 4 are shown in Figure 1A-C. FAM13A expression was significantly increased in the airway epithelia of patients with moderate to severe COPD (as defined by GOLD Stages 3 & 4) when compared to those from non-smoking controls (Figure 1D). After stratification of COPD patients by smoking status, epithelial-specific FAM13A expression was highest in current smokers when compared to non-smoking controls and moderately elevated in ex-smokers (Figure 1E). Interestingly, we observed a significant negative correlation between the epithelial-specific FAM13A expression and forced expiratory volume in one second (FEV1) to vital capacity (FVC) percent predicted (%) (Figure 1F), indicating that FAM13A expression is increased with decreasing lung function.

To determine the expression of several key regulatory genes associated with the TGFβ1 signaling pathway in human airway epithelial cells, gene expression data set was assessed in (N=68) healthy non-smokers, (N=101) smokers without COPD and (N=42) smokers with COPD (Table 2). TGFβ1 and COL1A1 gene expression were upregulated, whereas CDH1 and CTNNB1 were downregulated in the airway epithelium of smokers with COPD compared to healthy non-smokers (Figure 1G). FAM13A expression was positively associated with CTNNB1 and negatively associated with COL1A1 expression (p=3.79E-06; p=1.43E-09, respectively) (Figure 1H-I).

In a replication cohort (Table 3), current smokers exhibited decreased CTNNB1 expression compared to ex-smokers, irrespective of COPD status (p=0.002132) (Supplement figure 1A). After adjustment for age, sex and smoking status, subjects with COPD had decreased CTNNB1 expression compared to subjects without COPD (p=0.000867) (Supplement figure 1B). After adjustment for age, sex and smoking status, pre-bronchodilator FEV1 and FEV1/FVC were positively associated with expression of CTNNB1 (p=0.00087; p=0.00039, respectively) (Supplement figure 1C-D). Consistent with the cohort in Table 2, FAM13A expression was positively associated with CTNNB1 and negatively associated with COL1A1 expression (p=0.0091; p=0.000014, respectively) (Supplement figure 1E-F).

Collectively, these data provide clinical confirmation on the positive association of FAM13A with CTNNB1, and the negative association of FAM13A with COL1A1, in the airway epithelium of subjects with or without COPD.

FAM13A-/- cells exhibit increased COL1A1 and MMP2 expression in response to TGFβ1.

To determine the biological role of FAM13A in response to TGFβ1 stimulation, we used CRISPR-Cas9 gene editing (13) to negate FAM13A expression (FAM13A-/-) in 1HAEo cells. Sequencing of the targeted FAM13A genomic loci revealed indel frameshift mutations resulting in premature translational termination, with loss of FAM13A protein expression confirmed by a Western blot analysis (Figure 2A-C). Representative immunofluorescence images and quantitation showed an increase in COL1A1 protein expression in TGFβ1-treated FAM13A-/- cells when compared with the WT counterpart (Figure 3A-B). Real time PCR analyses consistently showed that FAM13A-/-...
cells mounted a greater induction in TGFβ1-mediated increase in COL1A1 gene expression compared to WT cells (Figure 3C). Similarly, FAM13A−/− cells exhibited increased MMP2 gene expression when compared to WT cells, and this increase was further elevated in response to TGFβ1 treatment (Figure 4A). The increases in MMP2 transcripts correlated well to increases in MMP2 protein expression as detected by Western blot analyses (Figure 4B-C). Taken together, these data showed that the loss of FAM13A expression in human airway epithelial cells enhances the transcriptional regulation of COL1A1 and MMP2 expression after TGFβ1 activation.

**Active CTNNB1 expression is elevated in FAM13A−/− cells and further induced by TGFβ1.**

Previously, it was reported that a loss of FAM13A increases the stability and expression of CTNNB1, which protects mice from chronic smoke-induced emphysema (27). Since CTNNB1 is also a transcriptional co-regulator that is involved in EMT (6), we determined whether FAM13A regulation of EMT genes such as COL1A1 and MMP2 occurs via modulation of CTNNB1. Expression of total CTNNB1 was upregulated in FAM13A−/− cells compared with WT, while stimulation with TGFβ1 further elevated the levels of CTNNB1 in FAM13A−/− cells (Figure 5A-B). In addition, active CTNNB1 levels were also upregulated in FAM13A−/− cells when compared to WT cells after TGFβ1 treatment (Figure 5C-D). When visualized by immunofluorescence imaging, total CTNNB1 expression localized in the cytoplasmic and plasma membrane compartments was increased in FAM13A−/− cells when compared to WT, and which was further elevated upon treatment with TGFβ1 (Figure 5E). Western blot quantification of cytoplasmic and nuclear fractions revealed a consistent increase in total and active CTNNB1 protein in FAM13A−/− cells treated with TGFβ1, but not in WT cells (Figure 5F-I). These data showed that a loss of FAM13A increases total and active CTNNB1 expression after TGFβ1 treatment.

**Inhibition of CTNNB1 reduces TGFβ1-induced increase in COL1A1 and MMP2 expression.**

To demonstrate the potential contribution of CTNNB1 in mediating TGFβ1-induced increase in COL1A1 and MMP2 expression, cells were pre-treated with CTNNB1 siRNA or with PKF118-310, a small molecule antagonist of the TCF4/CTNNB1 signaling complex (8), followed by 5 days of TGFβ1 treatment. CTNNB1 gene and protein expression were significantly reduced in CTNNB1 siRNA-treated groups compared to scrambled siRNA-treated groups in WT cells (Supplement Figure 2A-C) (https://figshare.com/s/73f1b275ead4bf8ed521). Treatment of WT cells with CTNNB1 siRNA reduced TGFβ1-induced increase in COL1A1 and MMP2 gene and protein expression (Figure 6A-D). Similarly, pharmacologic inhibition of TCF4/CTNNB1 complex using PKF118-310 reduced TGFβ1-induced increase in COL1A1 gene and protein expression (Figure 6E-F). PKF118-310 reduced TGFβ1-induced increase in MMP2 protein but not gene expression (Figure 6G-H). Next, to determine whether silencing CTNNB1 with siRNA impact COL1A1 in FAM13A−/− cells, we first confirmed that CTNNB1 siRNA treatment reduced CTNNB1 protein expression to below control levels in the presence or absence of TGFβ1, and recapitulated the induction of CTNNB1 protein by TGFβ1 but did not modify COL1A1 expression (Supplement Figure 2D-G). Collectively, these data showed that CTNNB1 is partially required in TGFβ1-induced increase in the expression of COL1A1 and MMP2.

**FAM13A over-expression reduces TGFβ1-induced increase in COL1A1 and MMP2 expression.**

Given that reduced FAM13A expression led to upregulation of COL1A1 and MMP2 expression via TGFβ1, we determined whether the over-expression of FAM13A reverses this phenomenon.
Initially, we used lipofectamine to transfect cells with FAM13A mRNA, and showed that FAM13A over-expression attenuated TGFβ1-induced increase in COL1A1 but not MMP2 gene expression (Supplement Figure 3A-C) (https://figshare.com/s/9d1590d6aba3fbe399e4). Due to its inherent toxicity and its ability for immune activation, lipofectamine has little clinical utility. Therefore, we next evaluated the therapeutic potential of FAM13A over-expression using clinically-validated LNP technology (34, 58). We encapsulated mRNA in LNP for cellular over-expression. Using flow cytometry to assess GFP+ cells as an indicator of transfection efficiency, we found that lipofectamine and LNP-based transfection resulted in comparable levels of efficiency (79.8% and 84.8% GFP+, respectively) when compared to untreated or GFP mRNA only controls (Supplement Figure 4A-E) (https://figshare.com/s/a2e989335106aef17b08). In addition, LNP uptake into cells was 100% as indicated by DiD-co-labeling (Supplement Figure 4F) (https://figshare.com/s/a2e989335106aef17b08).

As expected, FAM13A gene and protein expression was increased in cells treated with LNP-encapsulated FAM13A mRNA (Supplement Figure 5A-B) (https://figshare.com/s/c07494182a80d062bf4f). LNP-mediated transfection of cells with FAM13A mRNA led to reduced TGFβ1-induced increase in COL1A1 and MMP2 gene and protein expression (Figure 7A-D). TGFβ1 treatment significantly reduced both CDH1 gene and protein expression, this reduction was not further modified for cells transfected with FAM13A mRNA (Figure 7E-F). Total CTNNB1 protein expression was significantly reduced in cells treated with FAM13A mRNA when compared to untreated controls (Figure 7G). The combination of FAM13A mRNA and TGFβ1 treatment led to significant reduction of active CTNNB1 protein expression when compared to untreated controls (Figure 7H). Finally, we analyzed total and active CTNNB1 in cytoplasmic and nuclear fractions of 1HAEo cells treated with combinations of FAM13A and TGFβ1 for 3 days. FAM13A mRNA consistently prevented TGFβ1-induced increases in total and active cytoplasmic CTNNB1 (Figure 7I-J), as well as total nuclear CTNNB1 (Figure 7K), but not active nuclear CTNNB1 (Figure 7L). In summary, FAM13A over-expression attenuated TGFβ1-induced increase in the expression of COL1A1 and MMP2, concomitant with reduced expression of total and active CTNNB1.

**FAM13A influences cell morphology and skeletal organization of F-actin**

We compared the morphology of WT and FAM13A−/− cells transitioning from low to high density culture (Supplement Figure 6A-D) (https://figshare.com/s/7a59f19e8e6145c4bd5). While both cells retained an overall epithelial-like morphology, FAM13A−/− cells exhibited greater spreading compared to WT cells. Treatment with TGFβ1 induced both WT and FAM13A−/− cells to adopt elongated shapes at low density, while at high density, cells appeared to be less densely packed with enhanced spreading compared to non-treated cells. Higher resolution temporal imaging of non-TGFβ1 treated cells revealed lamellipodial protrusive activity that was enhanced in FAM13A−/− cells when compared to WT (Supplement video 1) (https://figshare.com/s/36d19e77b04d0a9df89). Cytoskeletal remodeling is associated with EMT (15), thus cells were stained with phalloidin to visualize filamentous actin (F-actin). At higher cell densities, cortical F-actin indicative of cell-cell contact was evident in both WT and FAM13A−/− cells (Figure 8A). FAM13A−/− cells treated with TGFβ1 resulted in reorganized F-actin that appeared less cortical and more like stress fibers (Figure 8A). This phenomenon was also observed in TGFβ1-treated WT cells, albeit at attenuated levels. After normalization to nuclei counts, the overall F-actin staining intensity was increased in FAM13A−/− but not in WT cells after TGFβ1
stimulation (Figure 8B). Cell packing density, assessed as number of nuclei per unit area at full cellular confluency, was intrinsically lower in FAM13A−/− compared to WT cells at baseline, and this effect was consistently decreased in both WT and FAM13A−/− cells after TGFβ1 treatment (Figure 8C). Taken together, FAM13A is involved in cytoskeletal organization of F-actin, and TGFβ1-induced changes in COL1A1 and MMP2 expression and cell packing density.

Discussion

In this study, we provide new data based on functional knockout of FAM13A expression in a human lung epithelial cell model designed to assess the role of FAM13A in the response to TGFβ1 stimulation, which highlights a negative causal relationship between FAM13A and CTNNB1 expression in airway epithelial cells. Specifically, we showed that a complete loss of FAM13A expression in lung epithelial cells enhances TGFβ1’s ability to increase the expression of COL1A1 and MMP2 by upregulating CTNNB1 expression, whereas over-expression of FAM13A attenuated the expression of COL1A1, MMP2 and CTNNB1. In analyses of airway epithelial cells from human subjects, we found that FAM13A protein expression was upregulated, while CTNNB1 gene expression was downregulated, in smokers with COPD compared to healthy non-smokers. Collectively, these data suggest that FAM13A is a modifier of TGFβ1-mediated signaling and its up-regulation in smokers at risk for COPD may protect the lungs against epithelial remodeling that leads to progressive narrowing of the airways in COPD.

Mass spectrometry studies of FAM13A-interacting proteins have implicated pathways enriched in “CTNNB1 phosphorylation cascade” and “CTNNB1 degradation by the destruction complex” (27). The proposed canonical mechanism by which TGFβ1 regulates extracellular matrix-related gene expression is through phosphorylation of Smad2/3, and their subsequent translocation to the nucleus to regulate transcription (38). In connecting the role of CTNNB1 in TGFβ1 signaling, Zhou and colleagues showed a direct interaction between Smad3 and CTNNB1 in the regulation of alpha smooth muscle actin (αSMA) after TGFβ1 stimulation (60). It was also reported that siRNA-mediated silencing of CTNNB1 reduces TGFβ1-induced matrix production in airway smooth muscle cells (6). Growth factors such as TGFβ1 play a critical role in the pathogenesis of airway remodeling in COPD (16) and asthma (17). TGFβ1 is a multifunctional cytokine that is stored in the extracellular matrix compartment in its inactive form (12). Latent TGFβ1 can be activated via oxidation (55) or enzymatically cleaved to release the active form of TGFβ1 for the activation of structural and inflammatory cells (3), thereby resulting in the production and secretion of extracellular matrix and tissue remodeling.

In search of novel therapeutic targets to reduce airway tissue remodeling, we turned to validating genes identified from COPD genome-wide association studies that are strongly associated with lung function with known biology. This is because the likelihood of success of such therapeutics in Phase II clinical trials is 50 to 100% greater compared to therapeutic targets that are disconnected from genetics (14, 22, 30). To determine the role of FAM13A and CTNNB1 in TGFβ1-induced EMT, we used CRISPR-Cas9 gene editing technology to generate 1HAEo cells deficient in both alleles of FAM13A. Corvol and colleagues showed that FAM13A knockdown with siRNA augmented TGFβ1-induced αSMA and vimentin gene expression, and F-actin fibre formation in A549 cells (15). We extend these findings by showing that FAM13A−/− 1HAEo cells exhibited increased expression of COL1A1, MMP2, CTNNB1 and F-actin, molecules known to
be involved in airway remodeling. It was reported that F-actin localizes at the margins of airway epithelial cells from non-smokers, while F-actin in cells from smokers and patients with COPD was distributed throughout the cytoplasm (39). In addition, TGFβ1 treatment of A549 cells also promoted the accumulation of cytoplasmic F-actin (29). Finally, to connect the regulatory role of CTNNB1, we showed that CTNNB1 siRNA treatment partially disrupted TGFβ1-induced increase in COL1A1 and MMP2 expression. Treatment with PKF118-310, an inhibitor of the interaction between T cell factor 4 (Tcf4) and CTNNB1 complex, consistently reduced COL1A1 and MMP2 expression. Collectively, these data provide a more comprehensive understanding in the potential role of FAM13A in the regulation of CTNNB1 and in TGFβ1 signaling (5, 57), supporting an important implication in the context of airway tissue remodeling in patients at risk of developing severe COPD.

Single nucleotide polymorphisms in FAM13A have previously shown a strong association with reduced lung function and with increased risk of COPD; however, to date, its functional role in the pathogenesis of COPD has been unclear. Previously, Jiang and colleagues reported an inverse relationship between FAM13A and CTNNB1 protein expression in human bronchial epithelial (16HBE) cell line, in mouse studies and, in whole lung tissues from subjects with and without COPD (27). Our human tissue staining data specifically revealed an increase in FAM13A protein in the airway epithelium of patients with COPD compared to non-smokers. Although it is tempting to speculate that FAM13A may play a role in enhancing airway tissue remodeling, our in vitro data indicate the contrary response, where FAM13A deficiency increased collagen expression via an increase in CTNNB1 upon stimulation with TGFβ1. This suggests that the increase in FAM13A expression observed in COPD airway epithelium may help to control excess TGFβ1-induced COL1A1 and MMP2 expression. The exact biological mechanism for the increase in FAM13A protein in COPD airway epithelium is unclear, but a previous study using an airway epithelial cell line showed that hypoxia increases FAM13A expression (61). Cigarette smoking is associated with increased risk of hypoxemia in patients with COPD (28, 54), which may partially explain the increase in FAM13A expression. Using lipid nanoparticle technology to encapsulate and deliver human FAM13A gene in 1HAEo cells, we showed that over-expression of FAM13A partially protects against TGFβ1-induced increase in COL1A1 and MMP2 expression. Lipofectamine-mediated delivery of plasmids have commonly been used for cellular over-expression of genes of interests in cell culture models; however, net positive charges of lipofectamine-type agents have little clinical utility owing to their increased toxicity (56) and the requirement to enter dividing cells for gene transcription. Lipid nanoparticles contain ionizable cationic lipids that bind to negatively-charged RNA or DNA and form a protective coat that is net neutral at physiologic pH. LNPs containing mRNA can directly release into the cytoplasm for immediate protein translation without integrating genetic information in our genome. Although DNA transfections are generally more stable than RNA transfections, RNA transfections present more diverse therapeutic options, as exemplified by adoption of the current RNA-based vaccines against COVID19 (7, 45). Collectively, these data suggest that FAM13A over-expression maybe a potential strategy for therapeutic intervention in reducing airway tissue remodeling of smokers at risk for development of COPD.

In the present report, we use an in vitro model to demonstrate that a deficiency in FAM13A in response to TGFβ1 activates genes involved in airway tissue remodeling. Limitations not addressed by this model include an ideal confirmation for the role of FAM13A in airway epithelial
remodeling *in-vivo* that should be investigated using tissue specific FAM13A KO generated in mice, and subjected to chronic cigarette smoke exposure. Furthermore, the contribution of other factors such as oxidative stress, local inflammatory response and microbial interactions that may synergistically enhance the TGFβ1 signaling response in the airway tissues of patients with COPD. The exact mechanism by which FAM13A is upregulated in the airway epithelium of patients with COPD is unknown and awaits resolution. However, we speculate that upregulation of FAM13A in the airway epithelium of healthy smokers without COPD may reduce CTNNB1 expression for a more protective response in controlling excess tissue remodeling. Undoubtedly, CTNNB1 (β-catenin) signaling contributes to lung repair/regeneration in response to various injuries (24, 31); therefore, more extensive studies accounting for multiple competing mechanisms at play are required to determine the value of over-expressing FAM13A in tissue- and disease-specific treatment.

In summary, this report shows that airway epithelial cells deficient in FAM13A loses its ability to sequester CTNNB1, and FAM13A is inversely related to the regulation of TGFβ1-mediated response in this *in vitro* model. Over-expression of FAM13A via LNP-mediated mRNA delivery partially reverses this effect *in-vitro*. Moreover, the up-regulation of FAM13A protein in the airway epithelium of patients at risk of COPD and those with mild COPD may offer increased protection against further TGFβ1-mediated tissue remodeling. This work provides important mechanistic insights in the role of FAM13A serving as potential therapeutic target and limiting fibrotic response in the airway epithelium of smokers at risk of severe COPD by down-regulating CTNNB1 that may intersect with TGFβ1-mediated tissue remodeling responses.

**Acknowledgments**

We thank Amrit Samra for processing the lung tissue samples for immunohistochemical staining, and Dean English for scanning the sections using the Aperio Imaging System.

AT is a recipient of a MITACS Accelerate fellowship award. PRC acknowledges support from the Canadian Institutes for Health Research (CIHR FDN 148469) and the NanoMedicines Innovation Network (NMIN), a Canadian Networks of Centres of Excellence (NCE) in nanomedicine. DW is supported by the Swiss National Science Foundation (#183923). JAK is supported by the NMIN Postdoctoral Fellowship Award in Gene Therapy. This study receives support from the Canadian Institute of Health Research (CIHR).
References

1. Leavitt B, Cullis P, Petkau T, Hill A, Wagner P, Kulkarni J. Compositions and systems comprising transfection-competent vesicles free of organic-solvents and detergents and methods related thereto [Online]. The University of British Columbia. https://patents.google.com/patent/WO2020077007A1/#patentCitations [2020].

2. Agusti A and Hogg JC. Update on the Pathogenesis of Chronic Obstructive Pulmonary Disease. *N Engl J Med* 381: 1248-1256, 2019.

3. Annes JP, Munger JS and Rifkin DB. Making sense of latent TGFbeta activation. *J Cell Sci* 116: 217-224, 2003.

4. Asano K, Shikama Y, Shoji N, Hirano K, Suzuki H, Nakajima H. Tiotropium bromide inhibits TGF-beta-induced MMP production from lung fibroblasts by interfering with Smad and MAPK pathways in vitro. *Int J Chron Obstruct Pulmon Dis* 5: 277-286, 2010.

5. Attisano L and Labbe E. TGFbeta and Wnt pathway cross-talk. *Cancer Metastasis Rev* 23: 53-61, 2004.

6. Baarsma HA, Menzen MH, Halayko AJ, Meurs H, Kerstjens HA, Gosens R. beta-Catenin signaling is required for TGF-beta1-induced extracellular matrix production by airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 301: L956-965, 2011.

7. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, Diemert D, Spector SA, Rouphael N, Creech CB, McGavigan J, Khetan S, Segall N, Solis J, Brosz A, Fierro C, Schwartz H, Neuzil K, Corey L, Gilbert P, Janes H, Follmann D, Marovich M, Mascola J, Polakowski L, Ledgerwood J, Graham BS, Bennett H, Pajon R, Knightly C, Leav B, Deng W, Zhou H, Han S, Ivarsson M, Miller J, Zaks T, Group CS. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N Engl J Med* 384: 403-416, 2021.

8. Beyer C, Reichert H, Akan H, Mallano T, Schramm A, Dees C, Palumbo-Zerr K, Lin NY, Distler A, Gelse K, Varga J, Distler O, Schett G, Distler JH. Blockade of canonical Wnt signalling ameliorates experimental dermal fibrosis. *Ann Rheum Dis* 72: 1255-1258, 2013.

9. Celli BR and Wedzicha JA. Update on Clinical Aspects of Chronic Obstructive Pulmonary Disease. *N Engl J Med* 381: 1257-1266, 2019.

10. Cho MH, Boutaoui N, Klanderma BJ, Sylvia JS, Ziniti JP, Hersh CP, DeMeo DL, Hunninghake GM, Litonjua AA, Sparrow D, Lange C, Won S, Murphy JR, Beaty TH, Regan EA, Make BJ, Hokanson JE, Crapo JD, Kong X, Anderson WH, Tal-Singer R, Lomas DA, Bakke P, Gulsvik A, Pillai SG, Silverman EK. Variants in FAM13A are associated with chronic obstructive pulmonary disease. *Nat Genet* 42: 200-202, 2010.

11. Cho MH, McDonald ML, Zhou X, Mattheisen M, Castaldi PJ, Hersh CP, DeMeo DL, Sylvia JS, Ziniti J, Laird NM, Lange C, Litonjua AA, Sparrow D, Casaburi R, Barr RG, Regan EA, Make BJ, Hokanson JE, Lutz S, Dudenkov TM, Farzadegan H, Hetmansi JB, Tal-Singer R, Lomas DA, Bakke P, Gulsvik A, Crapo JD, Silverman EK, Beaty TH, Nett Genetics IE, Investigators CO. Risk loci for chronic obstructive pulmonary disease: a genome-wide association study and meta-analysis. *Lancet Respir Med* 2: 214-225, 2014.

12. Chung KF. Cytokines in chronic obstructive pulmonary disease. *Eur Respir J Suppl* 34: 50s-59s, 2001.

13. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F. Multiplex genome engineering using CRISPR/Cas systems. *Science* 339: 819-823, 2013.
14. Cook D, Brown D, Alexander R, March R, Morgan P, Satterthwaite G, Pangalos MN. Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework. *Nat Rev Drug Discov* 13: 419-431, 2014.

15. Corvol H, Rousselet N, Thompson KE, Berdah L, Cottin G, Foussigniere T, Longchampt E, Fiette L, Sage E, Prunier C, Drumm M, Hodges CA, Boelle PY, Guillot L. FAM13A is a modifier gene of cystic fibrosis lung phenotype regulating rh0a activity, actin cytoskeleton dynamics and epithelial-mesenchymal transition. *J Cyst Fibros* 17: 190-203, 2018.

16. de Boer WI, van Schadewijk A, Sont JK, Sharma HS, Stolk J, Hiemstra PS, van Krieken JH. Transforming growth factor beta1 and recruitment of macrophages and mast cells in airways in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 158: 1951-1957, 1998.

17. Duvernelle C, Freund V and Frossard N. Transforming growth factor-beta and its role in asthma. *Pulm Pharmacol Ther* 16: 181-196, 2003.

18. Godinas L, Corhay JL, Henket M, Guiot J, Louis R, Moermans C. Increased production of TGF-beta1 from sputum cells of COPD: Relationship with airway obstruction. *Cytokine* 99: 1-8, 2017.

19. Gruenert DC, Basbaum CB, Welsh MJ, Li M, Finkbeiner WE, Nadel JA. Characterization of human tracheal epithelial cells transformed by an origin-defective simian virus 40. *Proc Natl Acad Sci U S A* 85: 5951-5955, 1988.

20. Hancock DB, Eijgelsheim M, Wilk JB, Gharib SA, Loehr LR, Marciande KD, Franceschini N, van Durme YM, Chen TH, Barr RG, Schabath MB, Couper DJ, Brusselle GG, Psaty BM, van Duijn CM, Rotter JJ, Uitterlinden AG, Hofman A, Punjabi NM, Rivadeneira F, Morrison AC, Enright PL, North KE, Heckbert SR, Lumley T, Stricker BH, O'Connor GT, London SJ. Meta-analyses of genome-wide association studies identify multiple loci associated with pulmonary function. *Nat Genet* 42: 45-52, 2010.

21. Heuberger J and Birchmeier W. Interplay of cadherin-mediated cell adhesion and canonical Wnt signaling. *Cold Spring Harb Perspect Biol* 2: a002915, 2010.

22. Hobbs BD, de Jong K, Lamontagne M, Bosse Y, Shrine N, Artigas MS, Wain LV, Hall IP, Jackson VE, Wyss AB, London SJ, North KE, Franceschini N, Strachan DP, Beaty TH, Hokanson JE, Crapo JD, Castaldi PJ, Chase RP, Bartz TM, Heckbert SR, Psaty BM, Gharib SA, Zanen P, Lammers JW, Oudkerk M, Groen HJ, Locantore N, Tal-Singer R, Rennard SI, Vestbo J, Timens W, Pare PD, Lattourelle JC, Dupuis J, O'Connor GT, Wilk JB, Kim WJ, Lee MK, Oh YM, Vonk JM, de Koning HJ, Leng S, Belinsky SA, Tesfaigzi Y, Manichaikul A, Wang XQ, Rich SS, Barr RG, Sparrow D, Litonjua AA, Bakke P, Gulsvik A, Lahousse L, Brusselle GG, Stricker BH, Uitterlinden AG, Ampleford EJ, Bleecker ER, Woodruff PG, Meyers DA, Qiao D, Lomas DA, Yim JJ, Kim DK, Hawrylukwicz I, Sliwinski P, Hardin M, Fingerlin TE, Schwartz DA, Postma DS, MacNee W, Tobin MD, Silverman EK, Boezen HM, Cho MH, Investigators CO, Investigators E, LifeLines I, Group SR, International CGNI, Investigators UKB, International CGC. Genetic loci associated with chronic obstructive pulmonary disease overlap with loci for lung function and pulmonary fibrosis. *Nat Genet* 49: 426-432, 2017.

23. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, Cherniack RM, Rogers RM, Sciurba FC, Coxson HO, Pare PD. The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med* 350: 2645-2653, 2004.

24. Hu Y, Ng-Blichfeldt JP, Ota C, Ciminieri C, Ren W, Hiemstra PS, Stolk J, Gosens R, Konigshoff M. Wnt/beta-catenin signaling is critical for regenerative potential of distal lung epithelial progenitor cells in homeostasis and emphysema. *Stem Cells* 38: 1467-1478, 2020.
25. Huber AH, Nelson WJ and Weis WI. Three-dimensional structure of the armadillo repeat region of beta-catenin. *Cell* 90: 871-882, 1997.

26. Ishikawa S, Ishimori K and Ito S. A 3D epithelial-mesenchymal co-culture model of human bronchial tissue recapitulates multiple features of airway tissue remodeling by TGF-beta1 treatment. *Respir Res* 18: 195, 2017.

27. Jiang Z, Lao T, Qiu W, Polverino F, Gupta K, Guo F, Mancini JD, Naing ZZ, Cho MH, Castaldi PJ, Sun Y, Yu J, Laucho-Contreras ME, Kobzik L, Raby BA, Choi AM, Perrella MA, Owen CA, Silverman EK, Zhou X. A Chronic Obstructive Pulmonary Disease Susceptibility Gene, FAM13A, Regulates Protein Stability of beta-Catenin. *Am J Respir Crit Care Med* 194: 185-197, 2016.

28. Kent BD, Mitchell PD and McNicholas WT. Hypoxemia in patients with COPD: cause, effects, and disease progression. *Int J Chron Obstruct Pulmon Dis* 6: 199-208, 2011.

29. Kim JH, Jang YS, Eom KS, Hwang YI, Kang HR, Jang SH, Kim CH, Park YB, Lee MG, Hyun IG, Jung KS, Kim DG. Transforming growth factor beta1 induces epithelial-to-mesenchymal transition of A549 cells. *J Korean Med Sci* 22: 898-904, 2007.

30. King EA, Davis JW and Degner JF. Are drug targets with genetic support twice as likely to be approved? Revised estimates of the impact of genetic support for drug mechanisms on the probability of drug approval. *PLoS Genet* 15: e1008489, 2019.

31. Kneidinger N, Yildirim AO, Callegari J, Takenaka S, Stein MM, Dumitrascu R, Bohla A, Bracke KR, Morty RE, Brusselle GG, Schermuly RT, Eickelberg O, Konigshoff M. Activation of the WNT/beta-catenin pathway attenuates experimental emphysema. *Am J Respir Crit Care Med* 183: 723-733, 2011.

32. Kulkarni JA, Darjuan MM, Mercer JE, Chen S, van der Meel R, Thewalt JL, Tam YYC, Cullis PR. On the Formation and Morphology of Lipid Nanoparticles Containing Ionizable Cationic Lipids and siRNA. *ACS Nano* 12: 4787-4795, 2018.

33. Kulkarni JA, Thomson SB, Zaifman J, Leung J, Wagner PK, Hill A, Tam YYC, Cullis PR, Petkau TL, Leavitt BR. Spontaneous, solvent-free entrapment of siRNA within lipid nanoparticles. *Nanoscale* 2020.

34. Kulkarni JA, Witzigmann D, Chen S, Cullis PR, van der Meel R. Lipid Nanoparticle Technology for Clinical Translation of siRNA Therapeutics. *Acc Chem Res* 52: 2435-2444, 2019.

35. Kulkarni JA, Witzigmann D, Leung J, van der Meel R, Zaifman J, Darjuan MM, Grisch-Chan HM, Thony B, Tam YYC, Cullis PR. Fusion-dependent formation of lipid nanoparticles containing macromolecular payloads. *Nanoscale* 11: 9023-9031, 2019.

36. Lamontagne M, Couture C, Postma DS, Timens W, Sin DD, Pare PD, Hogg JC, Nickle D, Laviolette M, Bosse Y. Refining susceptibility loci of chronic obstructive pulmonary disease with lung eQTLs. *PLoS One* 8: e70220, 2013.

37. Lutz SM, Cho MH, Young K, Hersh CP, Castaldi PJ, McDonald ML, Regan E, Mattheisen M, DeMeo DL, Parker M, Foreman M, Make BJ, Jensen RL, Casaburi R, Lomas DA, Bhatt SP, Bakke P, Gulsvik A, Crapo JD, Beatty TH, Laird NM, Lange C, Hokanson JE, Silverman EK. A genome-wide association study identifies risk loci for spirometric measures among smokers of European and African ancestry. *BMC Genet* 16: 138, 2015.

38. Massague J. TGF-beta signal transduction. *Annu Rev Biochem* 67: 753-791, 1998.

39. Milara J, Peiro T, Serrano A, Cortijo J. Epithelial to mesenchymal transition is increased in patients with COPD and induced by cigarette smoke. *Thorax* 68: 410-420, 2013.
40. Mittmann N, Kuramoto L, Seung SJ, Haddon JM, Bradley-Kennedy C, Fitzgerald JM. The cost of moderate and severe COPD exacerbations to the Canadian healthcare system. *Respir Med* 102: 413-421, 2008.

41. Obeidat M, Hao K, Bosse Y, Nickle DC, Nie Y, Postma DS, Laviolette M, Sandford AJ, Daley DD, Hogg JC, Elliott WM, Fishbane N, Timens W, Hysi PG, Kaprio J, Wilson JF, Hui J, Rawal R, Schulz H, Stubbe B, Hayward C, Polasek O, Jarvelin MR, Zhao JH, Jarvis D, Kahonen M, Franceschini N, North KE, Loth DW, Brusselle GG, Smith AV, Gudnason V, Bartz TM, Wilk JB, O'Connor GT, Cassano PA, Tang W, Wain LV, Soler Artigas M, Gharib SA, Strachan DP, Sin DD, Tobin MD, London SJ, Hall IP, Pare PD. Molecular mechanisms underlying variations in lung function: a systems genetics analysis. *Lancet Respir Med* 3: 782-795, 2015.

42. Perotin JM, Adam D, Vella-Boucaud J, Delepine G, Sandu S, Jonvel AC, Prevost A, Berthiot G, Pison C, Lebargy F, Birembaut P, Deslee G. Delay of airway epithelial wound repair in COPD is associated with airflow obstruction severity. *Respir Res* 15: 151, 2014.

43. Pillai SG, Ge D, Zhu G, Kong X, Shianna KV, Need AC, Hersh CP, Bakke P, Gulsvik A, Ruppert A, Lodrup Carlsen KC, Roses A, Anderson W, Rennard SI, Lomas DA, Silverman EK, Goldstein DB. A genome-wide association study in chronic obstructive pulmonary disease (COPD): identification of two major susceptibility loci. *PLoS Genet* 5: e1000421, 2009.

44. Pillai SG, Kong X, Edwards LD, Cho MH, Anderson WH, Coxson HO, Lomas DA, Silverman EK, Eclipse, Investigators I. Loci identified by genome-wide association studies influence different disease-related phenotypes in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 182: 1498-1505, 2010.

45. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, Perez JL, Perez Marc G, Moreira ED, Zerbini C, Bailey R, Swanson KA, Roychoudhury S, Koury K, Li P, Kalina WV, Cooper D, Frenc RW, Jr., Hammitt LL, Tureci O, Nell H, Schaefer A, Unal S, Tresnan DB, Mather S, Dormitzer PR, Sahin U, Jansen KU, Group CCT. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med* 383: 2603-2615, 2020.

46. Popova AP, Bozyk PD, Goldsmith AM, Linn MJ, Lei J, Bentley JK, Hershenson MB. Autocrine production of TGF-beta1 promotes myofibroblastic differentiation of neonatal lung mesenchymal stem cells. *Am J Physiol Lung Cell Mol Physiol* 298: L735-743, 2010.

47. Siedlinski M, Tingley D, Lipman PJ, Cho MH, Litonjua AA, Sparrow D, Bakke P, Gulsvik A, Lomas DA, Anderson W, Kong X, Rennard SI, Beaty TH, Hokanson JE, Crapo JD, Lange C, Silverman EK. Dissecting direct and indirect genetic effects on chronic obstructive pulmonary disease (COPD) susceptibility. *Hum Genet* 132: 431-441, 2013.

48. Steiling K, van den Berge M, Hijazi K, Florido R, Campbell J, Liu G, Xiao J, Zhang X, Duclos G, Drizik E, Si H, Perdomo C, Dumont C, Coxson HO, Alekseyev YO, Sin D, Pare P, Hogg JC, McWilliams A, Hiemstra PS, Sterk PJ, Timens W, Chang JT, Sebastiani P, O'Connor GT, Bild AH, Postma DS, Lam S, Spira A, Lenburg ME. A dynamic bronchial airway gene expression signature of chronic obstructive pulmonary disease and lung function impairment. *Am J Respir Crit Care Med* 187: 933-942, 2013.

49. Takizawa H, Tanaka M, Takami K, Ohtoshi T, Itó K, Satoh M, Okada Y, Yamasawa F, Nakahara K, Umeda A. Increased expression of transforming growth factor-beta1 in small airway epithelium from tobacco smokers and patients with chronic obstructive pulmonary disease (COPD). *Am J Respir Crit Care Med* 163: 1476-1483, 2001.

50. Tam A, Hughes M, McNagny KM, Obeidat M, Hackett TL, Leung JM, Shaipanich T, Dorschel DR, Singhera GK, Yang CWT, Pare PD, Hogg JC, Nickle D, Sin DD. Hedgehog
signaling in the airway epithelium of patients with chronic obstructive pulmonary disease. *Sci Rep* 9: 3353, 2019.

51. Tam A, Wadsworth S, Dorscheid D, Man SF, Sin DD. Estradiol increases mucus synthesis in bronchial epithelial cells. *PLoS One* 9: e100633, 2014.

52. van Amerongen R and Nusse R. Towards an integrated view of Wnt signaling in development. *Development* 136: 3205-3214, 2009.

53. Van Scoyk M, Randall J, Sergew A, Williams LM, Tennis M, Winn RA. Wnt signaling pathway and lung disease. *Transl Res* 151: 175-180, 2008.

54. Vos PJ, Folgering HT and van Herwaarden CL. Predictors for nocturnal hypoxaemia (mean SaO2 < 90%) in normoxic and mildly hypoxic patients with COPD. *Eur Respir J* 8: 74-77, 1995.

55. Wang RD, Wright JL and Churg A. Transforming growth factor-beta1 drives airway remodeling in cigarette smoke-exposed tracheal explants. *Am J Respir Cell Mol Biol* 33: 387-393, 2005.

56. Wang T, Larcher LM, Ma L, Veedu RN. Systematic Screening of Commonly Used Commercial Transfection Reagents towards Efficient Transfection of Single-Stranded Oligonucleotides. *Molecules* 23: 2018.

57. Warner DR, Greene RM and Pisano MM. Cross-talk between the TGFbeta and Wnt signaling pathways in murine embryonic maxillary mesenchymal cells. *FEBS Lett* 579: 3539-3546, 2005.

58. Witzigmann D, Kulkami JA, Leung J, Chen S, Cullis PR, van der Meel R. Lipid nanoparticle technology for therapeutic gene regulation in the liver. *Adv Drug Deliv Rev* 2020.

59. Xing Y, Takemaru K, Liu J, Berndt JD, Zheng JJ, Moon RT, Xu W. Crystal structure of a full-length beta-catenin. *Structure* 16: 478-487, 2008.

60. Zhou B, Liu Y, Kahn M, Ann DK, Han A, Wang H, Nguyen C, Flodby P, Zhong Q, Krishnaveni MS, Liebler JM, Minoo P, Crandall ED, Borok Z. Interactions between beta-catenin and transforming growth factor-beta signaling pathways mediate epithelial-mesenchymal transition and are dependent on the transcriptional co-activator cAMP-response element-binding protein (CREB)-binding protein (CBP). *J Biol Chem* 287: 7026-7038, 2012.

61. Ziolkowska-Suchanek I, Mosor M, Podralska M, Izykowska K, Gabryel P, Dyszkiewicz W, Slomski R, Nowak J. FAM13A as a Novel Hypoxia-Induced Gene in Non-Small Cell Lung Cancer. *J Cancer* 8: 3933-3938, 2017.
Table 1. Demographics of subjects with COPD: Immunohistological cohort study

| Characteristic            | Non-smoking controls | COPD GOLD STAGE 2 | COPD GOLD STAGE 3,4 |
|---------------------------|----------------------|-------------------|---------------------|
| Sex (M/F)                 | 2/3                  | 6/3               | 3/5                 |
| Smoking status (non/current/ex/NA) | 5/0/0/0             | 0/4/3/2          | 0/1/7/0            |
| Age, years (mean±SD)      | 59.6±19.8            | 63.7±9.0          | 61.0±6.1            |
| %FEV1/FVC (mean±SD)       | 83.0±4.4             | 57.1±5.6          | 33.4±11.9           |

Table 2. Demographics of non-smokers, smokers, and smokers with COPD for airway epithelial gene expression analyses (Cohort 1). Statistics are shown as either number (percentage) or median (interquartile range) where appropriate.

|                     | Non-smokers (N=68) | Smokers (N=101) | Smokers with COPD (N=42) |
|---------------------|--------------------|-----------------|--------------------------|
| Male                | 42 (61.8%)         | 68 (67.3%)      | 31 (73.8%)               |
| Female              | 26 (38.2%)         | 33 (32.7%)      | 11 (26.2%)               |
| Age                 | 39.0 (15.2)        | 43.0 (8.0)      | 51.5 (12.5)              |
| Pack Years          | 0.0 (0.0)          | 24.0 (22.0)     | 30.5 (26.5)              |

Table 3. Demographics of subjects with or without COPD for airway epithelial gene expression analyses (Cohort 2).

| GEO number GSE37147 | current smokers with COPD | current smokers without COPD | ex-smokers with COPD | ex-smokers without COPD |
|---------------------|---------------------------|------------------------------|----------------------|-------------------------|
| Total               | 30                        | 69                           | 57                   | 82                      |
| Sex (Female)        | 14 (46.7%)                | 35 (50.7%)                   | 21 (36.8%)           | 33 (40.2%)              |
| Sex (Male)          | 16 (53.3%)                | 34 (49.3%)                   | 36 (63.2%)           | 49 (59.8%)              |
| Age, years (mean±SD)| 63.2 (6.7)                | 62.2 (6.0)                   | 66.1 (5.6)           | 65.8 (5.0)              |
| %FEV1/FVC (mean±SD) | 56.9 (11.7)               | 74.6 (5.5)                   | 60.3 (7.5)           | 75.3 (5.7)              |
Figure Legends

Figure 1. FAM13A protein expression is up-regulated in the airway epithelium of patients with COPD. Representative images of paraffin-embedded human lung tissues from A) non-smoking control, B) COPD GOLD 2 and C) COPD GOLD 4 stained for FAM13A protein expression. D) Airway-specific FAM13A expression was quantified and normalized to length of basement membrane in µm. E) Airway-specific FAM13A expression was stratified by smoking status in patients with COPD (current vs. ex-smokers). F) Correlation between total epithelial-specific FAM13A expression (data log-transformed) with FEV1/FVC (%). Values were expressed as mean ± SEM. The Kruskal–Wallis test with Dunnett’s multiple comparisons test was used in panels D-E. (Note: one non-COPD subject has no reported FEV1/FVC ratio in panel F). In panel F, red dots=non-smoking controls, blue dots=COPD GOLD2 and orange dots=COPD GOLD3,4. G) Boxplots of TGFβ1, CTNNB1, COL1A1 and CDH1 gene expression in the small airway epithelium of (N=68) healthy non-smokers, (N=101) smokers without COPD and (N=42) smokers with COPD (box indicates the median and the interquartile range). Gene expression correlation analyses between H) CTNNB1 and FAM13A, and I) COL1A1 and FAM13A. The p-value was obtained using a linear regression model with gene expression as the response variable and adjusted for age, sex and smoking status.

Figure 2. Generation of FAM13A−/− 1HAEo cells using CRISPR-Cas9. A) Sequencing of the FAM13A genomic loci of WT 1HAEo cells and a FAM13A−/− clone generated using CRISPR-Cas9 targeting. Targeted guide RNA sequence indicated with black line; PAM recognition motif indicated with red. Alignment indicated a 2bp deletion in one allele and a net 14bp deletion in the other allele. B) Both frame-shifted alleles encode for predicted truncated proteins of 118 and 87 amino acids resulting from premature termination codons. Wildtype FAM13A is 697 amino acids long. C) Western blot analysis for FAM13A and β-actin expression of lysates from 1HAEo WT and FAM13A−/− cells.

Figure 3. Loss of FAM13A promotes increased COL1A1 protein expression in response to TGFβ1 stimulation. A) Representative confocal images of COL1A1 protein expression, with DAPI counterstaining for nuclei, in WT and FAM13A−/− cells with and without TGFβ1 treatment (1 week). Scale bar = 100µm. B) Assessment of COL1A1 protein intensity normalized to number of nuclei. C) Gene expression of COL1A1 normalized to GAPDH in WT and FAM13A−/− cells at baseline were expressed as fold change normalized to WT controls. Values were expressed as mean ± SEM (N=4 independent experiments). One-way analysis of variance with Bonferroni’s multiple comparisons test was used in panels B and C.

Figure 4. Loss of FAM13A promotes increased MMP2 expression in response to TGFβ1 stimulation. A) Assessment of fold change in expression of MMP2 normalized to GAPDH in WT and FAM13A−/− cells in the absence or presence of TGFβ1 treatment (1 week). B) Representative western blot and C) quantification of MMP2 protein expression normalized to β-actin in WT and FAM13A−/− cells in the absence or presence of TGFβ1 treatment. Values were expressed as mean ± SEM (N=4 independent experiments). One-way analysis of variance with Bonferroni’s multiple comparisons test was used in panels A and C.
Figure 5. Loss of FAM13A promotes increased total and active CTNNB1 expression in response to TGFβ1 stimulation. A) Representative western blot and B) quantification of total CTNNB1 protein expression normalized to β-actin in WT and FAM13A−/− cells with and without TGFβ1 treatment. C) Representative western blot and D) quantification of active CTNNB1 protein expression normalized to β-actin in WT and FAM13A−/− cells with and without TGFβ1 treatment. E) Representative confocal images of total CTNNB1 protein expression, with DAPI counterstaining for nuclei, in WT and FAM13A−/− cells with and without TGFβ1 treatment. Scale bar = 25µm. Western blot quantification of F) total CTNNB1 and G) active CTNNB1 cytoplasmic protein expression normalized to β-actin in WT and FAM13A−/− cells with and without TGFβ1 treatment. Western blot quantification of H) total CTNNB1 and I) active CTNNB1 nuclear protein expression normalized to HDAC2 in WT and FAM13A−/− cells with and without TGFβ1 treatment. Values were expressed as mean ± SEM (N=3-4 independent experiments). One-way analysis of variance with Bonferroni’s multiple comparisons test was used in panels B, D, and F-I.

Figure 6. Effect of CTNNB1 siRNA and pharmacologic inhibitor on TGFβ1-induced COL1A1 and MMP2 expression in 1HAEo cells
Wildtype 1HAEo cells were treated with control or CTNNB1 siRNA, in the absence or presence of TGFβ1 treatment, and assessed for A) COL1A1 gene expression relative to GAPDH, B) COL1A1 protein expression relative to β-actin, C) MMP2 gene expression relative to GAPDH, and D) MMP2 protein expression relative to β-actin. Wildtype 1HAEo cells were treated with control or 100ng/ml PKF118-310, in the absence or presence of TGFβ1 treatment, and assessed for E) COL1A1 gene expression relative to GAPDH, F) COL1A1 protein expression relative to β-actin, G) MMP2 gene expression relative to GAPDH, and H) MMP2 protein expression relative to β-actin. As shown are representative western blots and quantitation (B,D,F,H). Values were expressed as mean ± SEM (N=3 independent experiments). One-way analysis of variance with Bonferroni’s multiple comparisons test was used in all panels.

Figure 7. LNP-mediated over-expression of FAM13A decreases TGFβ1-induced increase in COL1A1 and MMP2 expression in 1HAEo cells.
Wildtype 1HAEo cells treated with empty LNP or LNP-FAM13A mRNA in the absence or presence of TGFβ1 treatment, and assessed for A) COL1A1 gene expression relative to GAPDH, B) COL1A1 protein expression relative to β-actin, C) MMP2 gene expression relative to GAPDH, D) MMP2 protein expression relative to β-actin, E) CDH1 gene expression relative to GAPDH, F) CDH1 protein expression relative to β-actin, and G) total CTNNB1 and H) active CTNNB1 protein expression relative to β-actin. Western blots shown are representative. 1HAEo cells treated with empty LNP or LNP-FAM13A mRNA in the absence or presence of TGFβ1 treatment (3 days), were also assessed for I-J) cytoplasmic total and active CTNNB1 relative to β-actin, and K-L) nuclear total and active CTNNB1 relative to β-actin. Values were expressed as mean ± SEM (N=3-4 independent experiments). One-way analysis of variance with Bonferroni’s multiple comparisons test was used in all panels.

Figure 8. TGFβ1-induced remodeling of F-actin is enhanced in FAM13A−/− cells.
A) Representative confocal images and B) quantification of fold change in F-actin intensity/nuclei normalized to WT control were shown in WT and FAM13A−/− control cells in the presence or absence of TGFβ1 treatment. Scale bar = 25µm. C) Quantification on the number of nuclei per unit area was shown in WT and FAM13A−/− control cells at full confluency in the presence or
absence of TGFβ1 treatment. Values were expressed as mean ± SEM (N=3 independent experiments). A parametric t-test was performed in panel B. One-way analysis of variance with Bonferroni’s multiple comparisons test was used in panel C.

Supplemental material available at:

URL: https://figshare.com/s/994cccba6440c02acab7
DOI: 10.6084/m9.figshare.14036300

URL: https://figshare.com/s/73f1b275ead4bf8ed521
DOI: 10.6084/m9.figshare.13058954

URL: https://figshare.com/s/9d1590d6aba3fbe399e4
DOI: 10.6084/m9.figshare.13058960

URL: https://figshare.com/s/a2e989335106aef17b08
DOI: 10.6084/m9.figshare.13058963

URL: https://figshare.com/s/c07494182a80d062fb4f
DOI: 10.6084/m9.figshare.14385302

URL: https://figshare.com/s/7a59f19ea8e6145c4bd5
DOI: 10.6084/m9.figshare.13058966

URL: https://figshare.com/s/36d19e77bfd40ab9df89
DOI: 10.6084/m9.figshare.14036318
A

WT

CTTGTCCTCCCATGTCGACTTCACTCTGCTCTCTTGGACTTGGCGGCGGCGG

PAM

FAM13A/- Allele 1

CTTGTCCTCCCATGCAGACTTCATCTGCTCTCTTGGACTTGGCGGCGGCGG

FAM13A/- Allele 2

CTTGTCCTCCCATGTTCGTC

B

Predicted protein length

WT 697a.a.

FAM13A/- Allele 1 118a.a.

FAM13A/- Allele 2 87a.a.

C

WT  FAM13A/-

150kDa

100kDa

75kDa

50kDa

42kDa

FAM13A

β-actin
