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POU2AF1 Functions in the Human Airway Epithelium To Regulate Expression of Host Defense Genes

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In the process of seeking novel lung host defense regulators by analyzing genome-wide RNA sequence data from normal human airway epithelium, we detected expression of POU domain class 2–associating factor 1 (POU2AF1), a known transcription cofactor previously thought to be expressed only in lymphocytes. Lymphocyte contamination of human airway epithelial samples obtained by bronchoscopy and brushing was excluded by immunohistochemistry staining, the observation of upregulation of POU2AF1 in purified airway basal stem/progenitor cells undergoing differentiation, and analysis of differentiating single basal cell clones. Lentivirus-mediated upregulation of POU2AF1 in airway basal cells induced upregulation of host defense genes, including MX1, IFIT3, IFITM, and known POU2AF1 downstream genes HLA-DRA, ID2, ID3, IL6, and BCL6. Interestingly, expression of these genes paralleled changes of POU2AF1 expression during airway epithelial differentiation in vitro, suggesting POU2AF1 helps to maintain a host defense tone even in pathogen-free condition. Cigarette smoke, a known risk factor for airway infection, suppressed POU2AF1 expression both in vivo in humans and in vitro in human airway epithelial cultures, accompanied by deregulation of POU2AF1 downstream genes. Finally, enhancing POU2AF1 expression in human airway epithelium attenuated the suppression of host defense genes by smoking. Together, these findings suggest a novel function of POU2AF1 as a potential regulator of host defense genes in the human airway epithelium. The Journal of Immunology, 2016, 196: 3159–3167.
Materials and Methods

Human airway epithelial cells

Airway epithelial cells were collected by brushing the epithelium using flexible bronchoscopy, as previously described (10). Subjects were recruited under a protocol approved by the Weill Cornell Medical College and Rockefeller University Institutional Review Boards, with written informed consent obtained from each volunteer before enrollment in the study.

The human airway basal cells (defined by expression of basal cell markers, lacking expression of ciliated and secretory cell markers and self-renewal and differentiation capacity) were either obtained from Lonza (Walkersville, MD) or isolated from brushed airway epithelial cells. The basal cell clones derived from a single cell were isolated with sterile cloning cylinders. The mucociliary differentiation of airway basal cells was induced on air-liquid interface (ALI), as described previously (11). The BCI-N51.1-immortalized human airway basal cell line has been reported previously (12). The effect of cigarette smoke on gene expression was assessed on ALI model with cigarette smoke extract (CSE) exposure, as described previously (13, 14).

Gene expression analysis of clinical samples

To assess normal POU2AF1 gene expression in vivo, RNA-Seq analysis was carried out on small airway epithelium (SAE, 10th to 12th order bronchi) from five healthy nonsmokers. The raw data have been deposited in the Sequence Read Archive (SRA) section of the National Center for Biotechnology Information database (SRP005411; http://www.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?study=SRP005411). Data of POU2AF1 and all transcription factors with expression level >0.125 reads per kb of exon per million mapped reads detection limit were extracted for comparison.

To assess the effect of smoking on POU2AF1 and its downstream gene expression in vivo, microarray data (Affymetrix HG-U133 plus 2.0 microarray) from two SAE cohorts (cohort I, 60 healthy nonsmokers, 72 healthy smokers; cohort II, 16 healthy nonsmokers, 20 healthy smokers) were used. The raw data used for expression assessment have been deposited in the Gene Expression Omnibus (GEO) site (77659; http://www.ncbi.nlm.nih.gov/geo/). POU2AF1 probe 1569675_at was used to confirm POU2AF1 expression change from the microarray study, SAE from randomly selected subjects (17 healthy nonsmokers, 21 healthy smokers) were used for TaqMan PCR validation.

To assess the expression of POU2AF1 downstream genes in undifferentiated human airway basal cells and differentiated airway epithelium in vivo, 12 large airway epithelial samples obtained by brushing and 5 purified large airway basal cells (all from healthy nonsmokers) were evaluated using Affymetrix HG-U133 plus 2.0 microarrays. The raw data are publicly available in the GEO site (77659; http://www.ncbi.nlm.nih.gov/geo/).

Lentivirus production and infection

Lenti-POU2AF1, lenti-red fluorescent protein (RFP; as a control), and lenti-KLF4 (as a control) expressing lentiviral plasmids were from Thermofluor Scientific (Pittsburgh, PA). Lenti-Osigin1 (as a control) was generated in our laboratory using standard methods. These vectors have some shared components, including IRES, GFP, basicidin S resistance gene, and WPRE. The replication-deficient lentiviruses were generated in 293A cells using compatible packaging vectors. The infectious titer of each virus was determined by flow cytometry using the MDM cell line. For infection of the basal cells, recombinant lentiviruses at an equal multiplicity of infection (MOI) were added with 6% CSE (3%) between days 0 and 14 from the basolateral side of the Transwell inserts. The medium was changed every 2 d, and each time a fresh CSE aliquot was thawed and diluted accordingly.

Lentivirus-infected ALI culture followed by CSE treatment

For infection of the basal cells on ALI cultures, recombinant lentiviruses at an equal MOI were added at the time of seeding the cells on Transwell inserts (ALI day −2). The following day, the infectious media were removed and the ALI culturing protocol continued, as described above. In all experiments, the cells were infected with a MOI that allowed for ~90% infection of the cells, as determined by GFP positivity. Cells were exposed to CSE (3%) between days 0 and 14 from the basolateral side of the Transwell inserts. The medium was changed every 2 d, and each time a fresh CSE aliquot was thawed and diluted accordingly.

TaqMan real-time PCR, staining, and Western analyses

Standard methods were used. Only TaqMan probes with best coverage were used (Life Technologies, Grand Island, NY). Abs are listed in Supplemental Table II.

Statistical analysis

A two-tailed Student t test was used for statistical analyses of all in vitro experiments. In all analyses, a p value <0.05 was deemed significant, unless specified. The analysis detail of microarray and RNA-seq was described in the corresponding section above.

Results

Identification of POU2AF1 as a human airway epithelium gene

RNA-Seq has low noise and high specificity and, importantly, provides quantitative information on mRNA transcript number (16). Unexpectedly, in the process of seeking novel host defense regulators in the human airway epithelium, we detected expression in airway epithelium RNA-Seq data of POU2AF1, a transcription cofactor generally regarded as lymphocyte restricted (4, 6) (Supplemental Fig. 1A). The expression of POU2AF1 in the human airway epithelium has an average of 6 reads per kb of exon on million mapped reads, close to the average expression level of the transcription factors in the airway epithelium (Supplemental Fig. 1B). Using a POU2AF1 Ab (17), we documented POU2AF1 expression at the protein level is expressed in human tracheobronchial epithelium, with enriched expression in intermediate cells with elongated morphology and some ciliated cells (Supplemental Fig. 1C).

Ruling out lymphocyte contamination as source of POU2AF1 expression

To rule out the possibility of lymphocyte contamination in the airway epithelium as a source of the POU2AF1 expression, we reasoned that gene expression of fully differentiated airway epithelium derived from pure airway basal cells should be free of lymphocyte transcripts. Tracheal epithelium were brushed from three healthy nonsmokers and cultured in a flask to purify and expand airway basal cells. These cells were negative for the B cell markers CD20 and...
CD79B, whereas positive for the basal cell markers KRT5 and TP63 (data not shown). The purified basal cells were then cultured on ALI to induce differentiation. By microarray analysis, the secretory cell marker gene SCGB1A1 (CC10) and ciliated cell marker gene DNAH5 were upregulated during differentiation over time (Fig. 1A), indicating successful mucociliary differentiation. The induction of differentiation was further supported by the cilia (β-tubulin) and secretory cell (SCGB1A1) staining (data not shown). The microarray data demonstrated that POU2AF1 was upregulated on ALI day 7, reaching a peak on ALI day 21 (Fig. 1B), slightly later than the peak of SCGB1A1 and DNAH5.

As lymphocytes might survive in the culture medium of airway epithelial cells (18), to further rule out minimum lymphocyte contamination, we induced airway epithelium differentiation from single cell–derived basal cell clones (Fig. 1C). Using Taq-Man analysis, we confirmed that the ALI cultures demonstrated upregulation of the secretory cell marker gene SCGB1A1, ciliated cell marker gene DNAH5, and POU2AF1 (Fig. 1D). Importantly, there was no expression of B cell markers CD20 and CD79B, nor the T cell marker CD3E (Fig. 1D). Finally, and consistent with these data, POU2AF1 was also upregulated in an immortalized airway basal cell line (12) during differentiation on ALI culture (Fig. 1E). Together, these data provide compelling evidence that POU2AF1 is not only expressed in human airway epithelium, but also upregulated during airway epithelium differentiation.

**Screening downstream genes of POU2AF1 in human airway epithelial cells**

Given that POU2AF1 binds to OCT1/OCT2 to exert its biologic effect, we assessed OCT1/OCT2 expression in the human airway epithelium. The RNA-Seq data demonstrated that expression levels of OCT1 and OCT2 were 28 and 17%, respectively, among all airway epithelial transcription factors. Western analysis confirmed that both OCT1 and OCT2 were expressed in human airway epithelial cells (data not shown). Consistent with these data, immunofluorescence staining showed both OCT1 and OCT2 localized in the nucleus of airway epithelial cells (data not shown).

**FIGURE 1.** Evidence that POU2AF1 expression in the airway epithelium is not secondary to lymphocyte contamination. (A and B) Upregulation of POU2AF1 during airway epithelium differentiation. Basal cells purified from brushed tracheal epithelial cells were cultured on an ALI to induce differentiation (n = 3 subjects, 6 time points). Gene expression over time was assessed by Affymetrix HG-U133 plus 2.0 microarrays. (A) Control expression of differentiation-specific genes; SCGB1A1 (secretory cells), DNAH5 (ciliated cells). (B) POU2AF1 expression. (C and D) POU2AF1 expression in ALI-differentiated cells derived from single clones of basal cells. Single clone–derived basal cells were generated and cultured on ALI to induce differentiation. Gene expressions were assessed by TaqMan PCR. (C) Single basal cell clone formation. One basal cell clone was tracked by phase microscopy from day 0 to 3. On day 8, the clone was stained with basal cell marker TP63 (green). Scale bar, 50 μm. (D) Gene expression change of single clone–derived basal cells on ALI culture (n = 8) compared with basal cells not plated on ALI (n = 3). Shown are expression of various genes in basal cells alone versus basal cells cultured on ALI. SCGB1A1 (secretory cells), DNAH5 (ciliated cells), and POU2AF1 are expressed in the differentiated cells derived from a single basal cell clone, but not basal cells. There is no expression of the B cell markers CD20 and CD79B, nor the T cell marker CD3E (Fig. 1D). Finally, and consistent with these data, POU2AF1 was also upregulated in an immortalized airway basal cell line (12) during differentiation on ALI culture (Fig. 1E). Together, these data provide compelling evidence that POU2AF1 is not only expressed in human airway epithelium, but also upregulated during airway epithelium differentiation.
As airway basal cells are undifferentiated and negative for POU2AF1 expression, we sought to use lentivirus-mediated POU2AF1 overexpression in basal cells as a sensitive way to screen for potential POU2AF1 downstream genes in airway epithelial cells. We achieved 90% gene transduction efficiency using lentiviral vectors (data not shown). There was strong POU2AF1 staining (both cytoplasm and nucleus) in lentivirus-POU2AF1-infected basal cells (data not shown). RNA-Seq was used to quantify the gene expression changes induced by POU2AF1. As a quality control to demonstrate that similar doses of gene delivery vehicles were used, we analyzed the expression levels of common components from the lentiviral vectors. The RNA-Seq data showed that whereas there were striking differences between POU2AF1 and RFP mRNA levels, expression of common components (IRES, GFP, blasticidin S resistance gene, WPRE) was similar between the lenti-POU2AF1 group and lenti-RFP group (Fig. 2A). TaqMan PCR of WPRE further confirmed this finding (Fig. 2B).

In addition to modulation of the IgG gene in B cells, POU2AF1 has multiple known downstream genes with diverse functions in lymphocytes, such as cellular metabolism, cell survival, surface receptors, ion transport, and cell adhesion (4–6). Many of these genes have roles in host defense. We reviewed the literature and made an up-to-date POU2AF1 downstream gene list (data not shown). Consistent with the literature (4–6, 19), POU2AF1 induced significant expression differences of known POU2AF1 downstream genes.
downstream genes compared with lenti-RFP and uninfected controls (Supplemental Fig. 2), including ID2 (19), ID3 (19), HLA-DRA (20), IL6 (21), S100A10 (22), and KCNN4 (22). Unsupervised hierarchical analysis of the top 50 genes upregulated by POU2AF1 clearly segregated the lenti-POU2AF1 group from the control groups (lenti-RFP and uninfected; Fig. 2C). Remarkably, gene ontology enrichment analysis revealed that immune response, response to biotic stimulus, and defense response categories (all \( p < 10^{-4} \)) were the major functions of the top 50 genes. Assessment of gene functions demonstrated that 74% (37 of 50) of the genes (Supplemental Table I, also highlighted in Fig. 2C) have host defense-related functions. These can be categorized as intracellular pathogen response (e.g., MX1, 144-fold; IFIT3, 79-fold; IFITM1, 32-fold); extracellular/surface host defense (e.g., CD53, 50-fold); cytokines and chemokines (CXCL10, 31-fold; IFNB1, 29-fold); complement (C1R, 23-fold); Ap presentation (HLA-DRA, 22-fold); and negative regulator of host defense response (USP18, 21-fold).

To validate the host defense genes induced by POU2AF1 at the protein level, Western analysis demonstrated host defense molecules MX1, IFIT3, and IFITM1 all were strikingly upregulated by POU2AF1 overexpression (Fig. 2D). In contrast, there is no expression difference of GFP, a common component of the lenti-RFP and lenti-POU2AF1 vectors, suggesting similar efficiency of gene transduction was achieved. To further exclude nonspecific effects (e.g., genetic background or unsuitable controls), we repeated POU2AF1 overexpression study in basal cells from a different subject. Two more unrelated lentivirus vectors (lenti-KLF4 and lenti-OSGIN1) were used as additional controls. The results of TaqMan PCR confirmed that POU2AF1 induced marked upregulation of host defense genes (Fig. 2E). Similar results were achieved from two additional independent experiments.

To further exclude confounding factors related to the lentivirus preparations, independent preparations of lenti-POU2AF1 and lenti-GFP (same expression cassette as lenti-POU2AF1, expressing GFP only) viruses were prepared and used to assess host defense gene induction. TaqMan PCR confirmed our previous finding that lenti-POU2AF1 can upregulate MX1, IFI44, IFI44L, and IFITM1 (data not shown). Collectively, these data suggest that POU2AF1 functions to broadly regulate host defense response genes in human airway epithelium.

**Upregulation of POU2AF1-regulated host defense genes during airway epithelium differentiation**

As POU2AF1 is upregulated during airway epithelium differentiation, we asked whether the host defense genes regulated by
POU2AF1 have orchestrated changes in gene expression. By comparing the expression profile of 12 brushed large airway epithelium (fully differentiated) versus 5 cultured large airway basal cells (undifferentiated), we found that more than half of the POU2AF1-regulated host defense genes had significantly higher expression in the differentiated airway epithelium and clearly divided the differentiated and undifferentiated airway epithelium into two populations (Fig. 3A). This observation was further assessed in the time course study of airway epithelium differentiation in vitro. In support of the microarray data, TaqMan PCR confirmed that MX1, HLA-DRA, IFIT3, IFI44, IFI44L, and IFITM1 were upregulated during airway epithelium differentiation over time (Fig. 3B). Consistently, Western analysis proved that there was striking upregulation of MX1, HLA-DRA, and IFITM1 during airway epithelium differentiation (Fig. 3C). Together, these data suggest that POU2AF1 is involved in maintaining a host defense tone in human airway epithelium.

Downregulation of POU2AF1 by cigarette smoking in human airway epithelium

Given that smoking is one of the major environmental factors affecting disease-related airway epithelium differentiation and a known insult having a deleterious effect on the lung host defense response, we postulated that expression of POU2AF1 might be regulated by cigarette smoking. To determine whether cigarette smoke has a direct effect on POU2AF1 expression, we used CSE to treat airway epithelium on ALI culture in vitro (13, 14). As a control, CSE induced upregulation of the oxidative stress gene CYP1A1 (Fig. 4A, left panel). Interestingly, POU2AF1 expression was suppressed by CSE in a dose-dependent manner (Fig. 4B, right panel). Consistently, immunofluorescence staining demonstrated that CSE treatment suppressed POU2AF1 expression in airway epithelium on ALI, which was accompanied with loss of mucociliary differentiation, as evidenced by the absence of β-tubulin IV (cilia marker) staining (Fig. 4B).

To assess the effect of smoking on POU2AF1 expression on the human airway in vivo, we used microarray to assess POU2AF1 expression changes in small airway epithelium from healthy nonsmokers and healthy smokers. Gene expression was assessed by microarray. Each dot represents one subject. The p value was calculated after Benjamini and Hochberg correction of genome-wide comparison. (C) POU2AF1 in small airway epithelium, microarray, initial cohort (60 healthy nonsmokers, 72 healthy smokers). (D) POU2AF1 in small airway epithelium, microarray, validation cohort (16 healthy nonsmokers, 20 healthy smokers). (E) TaqMan PCR validation of POU2AF1 expression of nonsmokers versus small airway epithelium. Data are from 17 healthy nonsmokers, 21 healthy smokers. (F and G) Principal component analysis of POU2AF1 downstream genes in the airway epithelium of nonsmokers and smokers. The list of the top 50 POU2AF1 downstream genes was from Fig. 3C. The study population was the same as that in Fig. 5C and 5D. (F) Principal component analysis, expression pattern of POU2AF1 and its downstream genes, initial cohort. (G) Principal component analysis, expression pattern of POU2AF1 and its downstream genes, validation cohort. Data represent mean ± SD. Statistics was calculated by two-tailed Student t test. **p < 0.01, ***p < 0.001, ****p < 0.0001.
expression in brushed airway epithelium on smokers compared with nonsmokers (23). Consistent with the in vitro finding, smoking caused significantly downregulated POU2AF1 expression in our initial cohort ($-1.8$-fold, $p < 10^{-4}$; Fig. 4C). The downregulation of POU2AF1 was unexpected as two known smoking-heightened pathways, NF-$k$B and the endoplasmic reticulum stress, are positive regulators of POU2AF1 expression in lymphocytes (24, 25). To verify these results, we assessed POU2AF1 expression in an independent microarray cohort of small airway epithelium (26), observing a 1.9-fold downregulation of POU2AF1 in smokers (Fig. 4D). Furthermore, using a different technique (TaqMan PCR), downregulation of POU2AF1 in the small airway epithelium by smoking was confirmed (3.4-fold downregulated, $p < 10^{-4}$; Fig. 4E).

We then asked whether POU2AF1-regulated host defense genes are globally deregulated by smoking. Interestingly, in both the initial and a validation airway epithelium microarray cohort (23, 26), expression of POU2AF1 and its downstream host defense genes segregated smokers and nonsmokers into two populations (Fig. 4F, 4G), suggesting smoking disorders the POU2AF1-regulated host defense gene network in the human airway epithelium. Based on these data, we conclude that, in addition to previous known stimuli (4, 6), POU2AF1 gene expression is also regulated by cigarette smoking, and impaired expression of POU2AF1 most likely contributes to the disordered airway host defense associated with smoking.

### Attenuation of smoking-induced downregulation of host defense genes by POU2AF1

Next, we assessed whether POU2AF1 can reverse the smoking-induced downregulation of host defense genes using ALI cultures with CSE treatment, with lentivirus vectors used to mediate sustained expression of POU2AF1 in the ALI cultures. We focused on three POU2AF1-regulated host defense genes, MX1, HLA-DRA, and IFITM1, all of which have good Abs available for detection of expression. As expected, CSE exposure suppressed expression of MX1 (Fig. 5A), HLA-DRA (Fig. 5B), and IFITM1 (Fig. 5C) at the mRNA level. Importantly, sustained POU2AF1 expression attenuated the smoking-induced downregulation of MX1 and HLA-DRA (Fig. 5A, 5B). Western analysis further confirmed these findings (Fig. 5D). For IFITM1, the attenuation was more obvious at the protein level than the mRNA level (Fig. 5C, 5D).

### Discussion

It is well recognized that exposure to cigarette smoke is a substantial risk factor for lung infection (27–29). Airway infection, in turn, can trigger exacerbations of smoking-induced lung diseases, such as chronic obstructive pulmonary disease (COPD), the third leading cause of death in the world (http://www.who.int/). Despite the accumulated epidemiologic observations, the mechanisms that mediate the smoking-induced dysfunctional host defense in the human airway epithelium have not been clearly defined. In the present human-based study, initiated from a genome-wide screening, POU2AF1 was identified as a novel host defense regulator in the human airway epithelium and that the impairment of host defense in the human airway epithelium induced by smoking is related, at least in part, to compromised expression of POU2AF1.

### Expression and regulation of POU2AF1 in human airway epithelium

POU2AF1 has been previously considered to be a lymphocyte-specific gene (4–9). It is constitutively expressed in B cells and is inducible in T cells upon stimulation (4). The stimuli that can upregulate POU2AF1 include IL-4, LPS, CD40 and BCR signaling (17), and PMA plus ionomycin (24). Sporadic literature, however, suggests POU2AF1 might have a role in cells other than lymphocytes. For example, in the murine intestinal follicle-associated epithelium, which includes M cells and is specialized for the uptake and transcytosis of macromolecules and microorganisms, there was higher POU2AF1 expression than in villous epithelium (30, 31). One common feature of these observations is that they are relevant to the interface between organs and the environment, which, together with the known functions, implies a defense role of POU2AF1.

Our genome-wide screening identified that POU2AF1 has an average expression level among all transcription factors in human airway epithelium. Although lymphocyte infiltration is common in airway epithelium (32), the upregulation of POU2AF1 during differentiation of airway basal cells and an airway basal cell immortalized line, as well as cells derived from simple basal cell clones, provided conclusive evidence that the observed POU2AF1 expression in human airway epithelium could not be due to lymphocyte contamination. The function and expression pattern of POU2AF1 are reminiscent of another airway transcription factor, ELF3, which is also upregulated during differentiation, not cell lineage specific, and related to innate immunity (33).

Airway epithelial cells and lymphocytes are usually localized in disparate milieus, which raise an intriguing question as to whether POU2AF1 has similar regulation mechanisms in both cell types. In the current study, we found that cigarette smoking is a novel repressor of POU2AF1 in human airway epithelium. Interestingly, microRNA miR-126, a negative regulator of POU2AF1 in the whole mouse airway (but in unknown cell types) (34), is upregulated by smoking in the human airway epithelium (35). These data suggest that, in response to smoking in vivo, both genetic and epigenetic mechanisms are most likely involved in the regulation of POU2AF1 in human airway epithelium. Finally, several
COPD-related cytokines and growth factors can differentially regulate POU2AF1, suggesting that the homeostasis of POU2AF1 is most likely disordered in COPD.

**Function of POU2AF1 in human airway epithelium**

POU2AF1 has been reported to regulate various genes, either in a direct or indirect manner. In lymphocytes, the gene expression regulated by POU2AF1 can be cell type or cell differentiation stage dependent (4). Besides IgG, the downstream genes of POU2AF1 in lymphocytes belong to multiple categories, including cellular metabolism, cell survival and proliferation, ion channel, cell adhesion, cytokines, and chemokines (4, 6).

Because POU2AF1 needs to bind to either OCT1 or OCT2 to take effect, we confirmed expression of OCT1 and OCT2 in human airway epithelium before assessing the functions of POU2AF1. Interestingly, we found many known POU2AF1 downstream genes expressed in B cells are also expressed in airway epithelium and regulated by POU2AF1. For example, ID2 is a transcription factor expressed in the distal lung tip epithelium (36); S100A10 (37) and KCNN4 (38) are ion channel-related genes, both of which have cystic fibrosis transmembrane conductance regulator-related functions in human airway epithelial cells; HLA-DRA is a MHC-II gene related to Ag presentation in human airway epithelium (39); and BCL6 is an inflammation suppressor in mouse airway epithelium (40). Together, these data suggest that the functions of POU2AF1 have some overlap between lymphocytes and airway epithelial cells.

Other than above known shared targets, the dominant function of the most highly induced genes by POU2AF1 in airway epithelial cells is related to host defense. Among the top 50 genes induced by POU2AF1, only HLA-DRA has been proved to be a downstream gene of POU2AF1 in lymphocytes. These genes function to defend against invasive pathogens through either directly suppressing pathogen survival/spreading (e.g., MX1, MX2, IFI44, IFI44L), or indirectly adjusting inflammatory cell responses (e.g., HLA-DRA, IL19, C1R). Many of these genes are involved in the IFN-mediated antiviral/microbe response, including two type I IFN genes, IFNB and IFNLF1, which are upregulated by POU2AF1. However, IFNG (type II IFN), a direct target gene of POU2AF1 in B cells (41), was not induced. As not all POU2AF1-modified genes are regulated by type I IFN, and OCT1/OCT2 binding sites are quite common in immunity-related genes (20, 21, 42), it is likely that upregulation of POU2AF1 enhanced an interactive network regulating host defense in human airway epithelium. Among all the lentiviral vectors (lenti-RFP, lenti-GFP, lenti-KLF4, lenti-OSG1N1) that we tested, only POU2AF1 induced such striking effects of regulation of host defense genes.

The role of POU2AF1 in regulating host defense genes is further supported by the finding that genes downstream of POU2AF1 have coordinated expression changes with POU2AF1 in human airway epithelium both in vitro and in vivo. Importantly, sustained expression of POU2AF1 attenuated the downregulation of host defense genes by cigarette smoke. Together, these data suggest that suppressed expression of POU2AF1 may contribute to the dysfunction of host defense induced by cigarette smoking in human airway epithelium. Besides mRNA, POU2AF1 can also regulate inflammation-related microRNAs in B cells (43). miR-146a is the only microRNA shown to be a direct target of POU2AF1 (43). miR-146a tunes inflammatory responses by targeting genes involved in the TLR pathway (43), an essential component of the host defense system (3). Interestingly, consistent with the downregulation of POU2AF1 by smoking, miRNA-146a is also expressed in human airway epithelium and downregulated by smoking in vivo (35).

One limitation of current study is that, because of technical challenges, we were not able to test the host defense response of the human airway epithelium in the context of suppression of POU2AF1.

In summary, our data demonstrate that expression of POU2AF1 is not only expressed in lymphocytes, but also in airway epithelium, where it is diminished by the stress of smoking. In the context that POU2AF1 is involved in the regulation of the host defense response of airway epithelium and that host defense dysfunction leads to vulnerability to respiratory infection, POU2AF1 and its related pathway might be therapeutic targets for smoking-related airway diseases.

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**Disclosures**

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