

Supplemental File

Microarray analysis identifies defect in regenerative and immune response pathways in COPD airway basal cells

Fanny Pineau¹, Gabriella Shumyatsky¹, Nicole Owuor¹, Nisha Nalamala¹, Sudhir Kotnala¹,
Sudhir Bolla¹, Nathaniel Marchetti¹, Steven Kelsen¹, Gerard J Criner¹, Uma S Sajjan^{1,2*}

Materials and methods

Airway epithelial cells

Basal cells were isolated from bronchial segments of normal donor lungs and explanted lungs from COPD patients at the time of lung transplantation as described previously (1, 2). Collection of the tissue was approved by Institutional Review Board of University Michigan, Ann Arbor, MI and Temple University, Philadelphia. For microarrays analysis tissue was collected from 8 healthy non-smokers and 8 COPD patients who had stopped smoking for at six months at the time of tissue collection. The basal cells were cultured in 12 mm transwells, as described previously (2, 3). Briefly, basal cells were cultured in collagen-coated 10 cm dishes in Bronchial Life medium (LifeLine Cell Technologies, Frederick, MD) until 80% confluent. Cells were harvested, flow-sorted based on the expression of nerve growth factor receptor (NGFR) to isolate basal cells. These cells were seeded in transwells at a density of 1×10^5 /well. Cells were grown under submerged conditions in Bronchial Life medium until confluent (5 to 7 days) and then maintained at air/liquid interface (ALI) for another 4 weeks to promote mucociliary differentiation. Cells were harvested either at a subconfluent stage that is 3-4 days after seeding (basal cells) or after four weeks of culturing at ALI (Mucociliary-differentiated cell cultures).

16HBE14o- human bronchial epithelial cells are immortalized normal bronchial cells. Cells were grown in Minimum Essential Medium (MEM) supplemented with 10% heatinactivated fetal bovine serum (FBS), and 2 mM of L-glutamine as described (4). Briefly cells were grown in Transwells and fed from both the basolateral and apical sides and these cells polarize and show transepithelial resistance of 800 to 1000 $\Omega \cdot \text{cm}^2$ within 3 to 4 days.

Transfection of airway epithelial cells

16HBE14o- cells were grown in transwells until they reached 90% confluency. Cells were then transfected with non-targeting or HOXB2 siRNA using siRNAmix Lipofectamine (ThermoFisher Scientific, Waltham, MA) and incubated for 6 hours as described previously (5). The transfection medium was replaced with fresh cell culture medium and incubation continued for another 2 days.

RNA and microarray processing

Cells were washed once with PBS, lysed in TRIZOL and total RNA was isolated by using miRNeasy kit (Qiagen, Valencia, CA) involving DNase treatment step. The quality and concentration of each RNA samples was assessed with Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA). Biotinylated cDNAs were prepared according to the Affymetrix Plus WT kit protocol (GeneChip® WT Plus Reagent Kit Manual P/N 703174 Rev. 2) from 400 ng total RNA and hybridized to Human Gene 2.1 ST arrays at 48°C. The arrays were washed, stained and scanned using the Affymetrix GeneTitan system (software version 4.3.0.1592). The LIMMA (Linear Models for Microarray Data) methodology was applied to the log₂-transformed expression data to identify differentially expressed genes (DEG) for each comparison. Differentially expressed genes were identified based on statistical significance (p-value of < 0.05 and up/down regulated by more than 2-fold) and further analyzed. Normalized data and raw data are available in Gene Expression Omnibus (GEO) with accession number GSE137557.

Gene Ontology

Gene Ontology (GO) and KEGG pathways were analyzed with WebGestalt (WEB-based Gene Set Analysis Toolkit) (6) using the Benjamini-Hochberg correction for multiple testing (FDR 5%). For Gene Ontology, only Biological Process terms are discussed because Cellular Component and Molecular Function terms were less relevant.

Flow cytometry

Mucociliary-differentiated cells were dissociated with accutase (ThermoFisher Scientific, Waltham, MA), cells were fixed with 4% paraformaldehyde, and incubated in PBS containing 1% BSA and 0.5% saponin to permeabilize and block the cells simultaneously. Cells were then incubated with acetylated tubulin (Sigma Aldrich, St. Louis, MO), Muc5AC (Abcam, Cambridge, MA) or TP63 (Abcam), bound antibodies were detected by using Alexafluor-labeled second antibodies. The cells were then analyzed in FACSCalibur Flow cytometer (BD Biosciences, San Jose, CA). The data was analyzed by FlowJo version 10 (Tree Star, Ashland, OR).

Transepithelial resistance

Transepithelial resistance of airway epithelial cell cultures maintained at air/liquid interface (ALI) for two weeks or 16HBE14o- cells cultured in transwells for 4 days was determined by using EVOM volt/Ohm meter (World Precision Instruments, Sarasota, FL) with EnOhm chambers as previously described (4).

Histology

For histological evaluation, cell cultures were fixed in buffered formalin, embedded in paraffin, 5 μ thick sections were deparaffinized and stained with hematoxylin and eosin (H and E) or periodic acid Schiff's (PAS) reagent (1).

Real-time PCR

cDNAs were synthesized from 500 ng of total RNA using LunaScript RT SuperMix kit (New England Biolabs, Ipswich, MA). Expression of *ADAMTSL3*, *ELF5*, *HOXA1*, *HOXB2*, *IVL*,

KRTDAP, *STC2*, *ELF5*, *VGLL1*, *MUC5AC* and *FOXJ1* was determined by using a primetime probe based realtime PCR assays (Integrated DNA Technologies, Coralville, IA). The expression levels of each gene is presented as fold change over house-keeping gene, glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*).

Immunofluorescence staining of cell cultures and lung sections.

Cells growing in the transwells were washed with PBS and fixed in ice cold methanol at 5 min (for occludin) or in 4% paraformaldehyde followed by permeabilization with 0.1% Triton X-100 in PBS for 15 min (for E-cadherin) (4, 5, 7). The cells were then blocked with 1% BSA and incubated in 1:50 (for E-cadherin) or 1:100 (for occludin) (both antibodies are purchased from ThermoFisher Scientific) overnight at 4° C. The bound antibody was detected by using appropriate Alexafluor-conjugated second antibodies, counter stained with DAPI to visualize nuclei and imaged under Zeiss Confocal microscope.

Lung tissue at second and third branching of bronchi was collected from 6 COPD and 6 healthy non-smokers under the approval of Temple Institute Review Board. All the COPD subjects were at end stage disease and undergoing lung transplantation. The tissues were fixed in 10% buffered formaldehyde and embedded in paraffin. Five micron thick lung sections were deparaffinized, subjected to antigen retrieval by boiling in sodium citrate buffer and endogenous peroxidase activity was quenched with hydrogen peroxide. The sections were permeabilized with 0.3% TRITON X-100, blocked with 5% normal horse serum and incubated with HOXB2 antibody (Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA) for 16 h at 4°C. Unbound antibody was removed, washed and then incubated with a second antibody conjugated with HRP (ImmPRESS HRP anti-mouse or anti-rabbit IgG, Vector Laboratories, Burlingame,

CA). Sections were then incubated with tyramide conjugated with Alexa Fluor 488 (Thermo Fisher Scientific), counter stained with DAPI and imaged using a fluorescence microscope.

Western blot analysis

After relevant treatment, cells were washed with cold PBS and lysed in RIPA buffer containing protease and phosphatase inhibitors. Equal amount of protein was subjected to Western blot analysis with antibodies to E-cadherin (abcam), occludin (BD Biosciences), HOXB2 or β -actin (Sigma Aldrich). Specific bands were quantified by densitometry using NIH image J and expressed as fold change over β -actin or over respective total protein.

Statistical analysis

To validate the DEGs identified by microarray analysis cells obtained from 8 normal and 8 COPD subjects were used in most of the experiments. The data was expressed as median with range and the statistical significance was assessed by non-parametric analysis, Mann-Whitney test to compare two groups and ANOVA on Ranks with Kruskal Wallace non parametric test to compare three groups. Other experiments were repeated at least 3 times in duplicates and the data presented as mean \pm SD and statistical significance was assessed by unpaired t test. A p-value of ≤ 0.05 was considered as statistically significant.

References

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Figure Legends

Supplemental Figure 1. Correlation of tissue developmental genes with polarization and differentiation markers. Total RNA or protein was extracted from 8 normal and 8 COPD patients at 1 (basal cells), 3 (polarized cells) and 5 (differentiated cells) weeks of culturing. mRNA expression of ELF5, VGLL1 and KRTDAP was assessed in basal cells and correlated with the expression of MUC5AC and FOXJ1 mRNA in differentiated cells (A and B). TER was determined at 3 weeks of culturing and correlated with the expression of ELF5, VGLL1 and KRTDAP. Data represents median with range from 8 COPD and 8 normal subjects and statistical significance was determined by Mann-Whitney test.

Supplemental Table 1. Study population

Cells used for microarrays

Subject	Age	Sex	Pack-years	FEV1% predicted
Normal (8)	50	4:4		
N4	59	F	-	-
N17	33	M	-	-
N18	16	M	-	-
N19	50	F	-	-
N20	52	M	-	-
N22	62	M	-	-
N24	50	F	-	-
N27	48	F	-	-
COPD (8)	54	5:3	41.25	17
C6	49	F	25	17
C10	53	F	37.5	14
C11	59	M	70	22
C12	49	F	136	13
C14	55	M	25	17
C15	45	M	31.25	17
C18	67	M	90	20
C19	64	M	45	29

Tissues used for immunohistochemistry

Normal (4)				
1201	70	M	-	-
1220	67	M	-	-
1283	47	F	-	-
1316	67	F	-	-
COPD (4)				
713	73	M	N/A	21
1193	58	F	N/A	43
1209	75	M	N/A	22
1213	59	F	N/A	18

N/A: Not available

Supplementary Table 1. Differentially expressed probes between COPD and normal basal cells

ID	C Avg (log ₂ N Avg)	log ₂ C Standard	N Standard	Fold Change	P-val	FDR P-val	Public Gene Symbol	Gene Description	Chromosome	Strand	Start	Stop
17056098	1.51	3.67	0.7	0.5 ↓	-4.47	1.79E-06	0.0496 NM_0055:HOXA1	homeobox A1	chr7	-	27132612	27135625
16678496	4.92	3.78	0.41	0.38 ↑	2.21	2.06E-06	0.0496 NM_1750:HIST3H2BB	histone cluster 3, H2bb	chr1	+	2.29E+08	2.29E+08
16991948	6.98	8.13	0.45	0.28 ↓	-2.21	3.28E-06	0.0527 NM_0010:TMEM2	teneurin transmembrane protei	chr5	+	1.67E+08	1.68E+08
17023799	2.93	4.61	0.57	0.65 ↓	-3.19	2.02E-05	0.0814 NM_1451:SLC2A12	solute carrier family 2 (facilitate	chr6	-	1.34E+08	1.34E+08
17085190	3.37	1.98	0.57	0.44 ↑	2.63	2.05E-05	0.0814		chr9	+	42037464	42037558
17085457	3.37	1.98	0.57	0.44 ↑	2.63	2.05E-05	0.0814		chr9	+	67864533	67864627
17094376	3.37	1.98	0.57	0.44 ↑	2.63	2.05E-05	0.0814		chr9	-	46669492	46669586
17107309	5.06	6.67	0.61	0.57 ↓	-3.05	4.72E-05	0.1382 NM_0162:VGLL1	vestigial-like family member 1	chrX	+	1.36E+08	1.36E+08
16820820	5.12	4.08	0.28	0.48 ↑	2.05	0.0001	0.1557 NR_00307:SNORD111	small nucleolar RNA, C/D box 11	chr16	+	70571908	70572001
16913627	1.75	3.21	0.51	0.72 ↓	-2.76	0.0001	0.1557 NM_0011:ARHGAP40	Rho GTPase activating protein 4C	chr20	+	37230577	37279678
17100201	5.23	6.48	0.59	0.66 ↓	-2.38	0.0002	0.1636 NM_0046:CLIC3	chloride intracellular channel 3	chr9	-	1.4E+08	1.4E+08
16846218	1.89	3.29	0.31	0.93 ↓	-2.64	0.0002	0.1661 NM_0021:HOXB2	homeobox B2	chr17	-	46618256	46623441
17121722	8	9.26	0.46	0.71 ↓	-2.39	0.0002	0.183		TCONS_I2_+		1	1201
16980051	3.83	2.19	0.8	0.5 ↑	3.12	0.0003	0.2061 NM_0011:CLGN	calmegin	chr4	-	1.41E+08	1.41E+08
16884607	2.76	3.76	0.45	0.69 ↓	-2	0.0003	0.2065 NM_0122:IL36RN	interleukin 36 receptor antagoni	chr2	+	1.14E+08	1.14E+08
17016509	5.33	4.08	0.44	0.58 ↑	2.39	0.0003	0.2065 ENST0000:HIST1H3J	histone cluster 1, H3j	chr6	-	27858093	27858588
16734355	2.59	3.99	0.63	0.88 ↓	-2.64	0.0003	0.2099 NR_00219:H19; MIR675	H19, imprinted maternally expre	chr11	-	2016406	2022700
17016366	6.02	4.51	0.61	0.51 ↑	2.84	0.0004	0.2109 NM_0035:HIST1H2AB	histone cluster 1, H2ab	chr6	-	26033320	26033796
16660014	3.31	4.76	0.63	0.76 ↓	-2.73	0.0005	0.2358 NM_0133:PADI1	peptidyl arginine deiminase, typ	chr1	+	17531621	17527501
16760953	4.49	6.31	0.68	0.85 ↓	-3.53	0.0005	0.2506 NM_0012:MFAP5	microfibrillar associated protein	chr12	-	8789942	8815484
16671514	1.21	2.84	0.53	0.84 ↓	-3.08	0.0007	0.2558 NR_03621: MIR4258	microRNA 4258	chr1	+	1.55E+08	1.55E+08
17121866	6.69	8.05	0.51	0.84 ↓	-2.57	0.0008	0.2692		TCONS_I2_+		1	1829
16773260	5.06	7.23	1.26	1.4 ↓	-4.48	0.0008	0.2692 NM_0011:ATP12A	ATPase, H+/K+ transporting, non	chr13	+	25254549	25285923
16873528	1.14	2.27	0.49	0.66 ↓	-2.19	0.0009	0.2692 XR_24400:LOC645553	uncharacterized LOC645553	chr19	-	46692287	46706319
16669278	2.21	3.23	0.4	0.6 ↓	-2.02	0.0009	0.2747 NM_0177:FAM46C	family with sequence similarity 4	chr1	+	1.18E+08	1.18E+08
16882834	4.2	6.31	0.73	1.75 ↓	-4.32	0.001	0.2747 NM_0023:MAL	mal, T-cell differentiation protei	chr2	+	95691422	95719737
16874683	4.47	5.9	0.77	0.84 ↓	-2.68	0.0011	0.2797 NM_0010:KLK5	kallikrein related peptidase 5	chr19	-	51446559	51456344
16746217	1.13	2.27	0.52	1.16 ↓	-2.2	0.0011	0.2815		chr11	-	1.3E+08	1.31E+08
16730157	1.25	2.59	0.31	1.16 ↓	-2.53	0.0011	0.2887 NM_0010:HEPHE1	hephaestin-like 1	chr11	+	93754378	93847374
17044485	2.04	3.06	0.63	0.25 ↓	-2.04	0.0012	0.294 NR_03836:HOTAIRM1	HOXA transcript antisense RNA, i	chr7	+	27135266	27139877
16842403	4.86	6.43	0.62	0.79 ↓	-2.97	0.0012	0.2981 NM_0010:LGALS9B	lectin, galactoside-binding, solub	chr17	-	20352708	20372296
17127212	3.18	4.29	0.55	0.39 ↓	-2.15	0.0016	0.3226		TCONS_I2_+		1	507
16737282	3.84	4.92	0.57	0.93 ↓	-2.12	0.0022	0.3422 NM_0012:ELF5	E74-like factor 5 (ets domain tra	chr11	-	34499855	34535352
16874702	7.42	8.45	0.5	0.93 ↓	-2.05	0.0024	0.3483 NM_0012:KLK7	kallikrein related peptidase 7	chr19	-	51479729	51487320
16816962	3.45	4.65	0.72	0.6 ↓	-2.31	0.0027	0.3578 NM_0010:SCNN1G	sodium channel, non voltage gat	chr16	+	23194036	23228204
16861126	1.96	3.94	0.55	1.93 ↓	-3.96	0.0028	0.3578 NM_0012:UPK1A	uroplakin 1A	chr19	+	36157715	36169367
17108856	6.59	4.99	0.6	1.52 ↑	3.03	0.003	0.3578 XR_92127:LOC1019301	zinc finger protein 839-like	chrX	-	3771051	3804591
16765080	6.28	7.86	1.1	0.94 ↓	-2.99	0.0031	0.3578 NM_0022:KRT4	keratin 4, type II	chr12	-	53200327	53208335
16735727	1.19	2.33	0.47	0.84 ↓	-2.21	0.0031	0.3578		chr11	-	9865362	9865423
17121738	5.31	6.36	0.63	0.65 ↓	-2.06	0.0032	0.3589		TCONS_I2_+		1	1110
16671061	1.39	2.96	0.52	1.24 ↓	-2.96	0.0038	0.3693 NM_0010:KPRP	keratinocyte proline-rich protein	chr1	+	1.53E+08	1.53E+08
17125596	6.98	5.6	0.41	1.24 ↑	2.6	0.0039	0.3693		TCONS_I2_+		1	318
16764907	0.89	2	0.63	0.85 ↓	-2.16	0.0039	0.3693 NM_1730:KRT6C	keratin 6C, type II	chr12	-	52862300	52867569
16972229	6.24	4.24	1.33	1.16 ↑	3.99	0.004	0.3693 NM_0071:ANXA10	annexin A10	chr4	+	1.69E+08	1.69E+08
16693393	3.14	4.44	0.95	0.91 ↓	-2.46	0.0042	0.3693 NM_0203:PGLYRP3	peptidoglycan recognition protei	chr1	-	1.53E+08	1.53E+08
16966621	4.67	5.87	0.78	1.06 ↓	-2.29	0.0043	0.3726 NM_0012:CWH43	cell wall biogenesis 43 C-termina	chr4	+	48988264	49064098
16711390	0.98	2.09	0.36	0.94 ↓	-2.15	0.0048	0.3794 NM_0174:CALML5	calmodulin-like 5	chr10	-	5540658	5541533
17002429	4	5.12	1.28	0.81 ↓	-2.18	0.005	0.3843 NM_0251:ATP10B	ATPase, class V, type 10B	chr5	-	1.6E+08	1.6E+08
16871403	4.83	6.29	1.26	1.64 ↓	-2.74	0.0054	0.3865 NM_0011:SBSN	suprabasin	chr19	-	36014269	36019253
17121736	3.89	5.13	0.67	0.97 ↓	-2.37	0.0059	0.3939		TCONS_I2_+		1	2334
17005862	2.7	1.62	0.26	0.83 ↑	2.13	0.006	0.3939 NM_0035:HIST1H3H	histone cluster 1, H3h	chr6	+	27777842	27778314
16872777	4.43	6.56	1.09	2.27 ↓	-4.39	0.007	0.4035 NM_0324:CNFN	cornifelin	chr19	-	42891171	42894444
16874751	3.03	5.24	0.98	2.05 ↓	-4.65	0.0071	0.4035 NM_0195:KLK12	kallikrein related peptidase 12	chr19	-	51532348	51538486
17125236	3.7	2.38	1	0.84 ↑	2.5	0.0078	0.4138		TCONS_I2_+		1	397
17125594	6.53	5.33	0.52	1.09 ↑	2.3	0.0081	0.4138		TCONS_I2_+		1	412
17079346	3.74	4.77	0.68	0.51 ↓	-2.04	0.0086	0.4144		chr8	-	96691066	96691143
17119706	3.74	4.77	0.68	0.51 ↓	-2.04	0.0086	0.4144		tc5302189+		1	78
16671094	3.92	5.1	1.03	1.1 ↓	-2.27	0.0093	0.4179 NM_0055:IVL	involucrin	chr1	+	1.53E+08	1.53E+08
16959505	1.71	0.71	0.7	0.65 ↑	2.01	0.01	0.4261		chr3	-	1.35E+08	1.35E+08
16671023	5.35	7.13	1.09	1.7 ↓	-3.43	0.0112	0.4363 NM_0190:CRCT1	cysteine rich C-terminal 1	chr1	+	1.52E+08	1.52E+08
16871349	2.47	3.59	0.6	1.57 ↓	-2.17	0.0114	0.4387 NM_0012:KRTDAP	keratinocyte differentiation-asso	chr19	-	35978226	35986460
16799739	6.73	4.83	1.32	0.45 ↑	3.72	0.0118	0.4445 NM_0011:CHAC1	ChaC glutathione-specific gamm	chr15	+	41244055	41248717
16670681	2.16	3.21	0.49	1.02 ↓	-2.06	0.0119	0.446 NM_0035:ANXA9	annexin A9	chr1	+	1.51E+08	1.51E+08
16840799	2.32	4.46	1.27	1.49 ↓	-4.41	0.0125	0.4492 NM_0011:ALOX12B	arachidonate 12-lipoxygenase, 1	chr17	-	7975954	7991022
17094586	3.77	2.71	0.9	0.89 ↑	2.1	0.0127	0.4527		chr9	-	69821760	69821841
16693383	3.18	4.21	0.95	0.95 ↓	-2.03	0.0134	0.4584 NM_0528:PGLYRP3	peptidoglycan recognition protei	chr1	-	1.53E+08	1.53E+08
16874722	2.66	3.77	0.6	0.9 ↓	-2.15	0.0136	0.4592 NM_0123:KLK9; KLK8	kallikrein related peptidase 9; k	chr19	-	51505664	51512890
16743593	4.66	6.05	1.69	0.99 ↓	-2.62	0.0141	0.462 NM_0046:TRPC6	transient receptor potential cati	chr11	+	1.01E+08	1.02E+08
16949261	3.18	2.17	0.44	0.54 ↑	2.01	0.0143	0.4631		chr3	+	1.86E+08	1.86E+08
17055551	2.73	1.72	0.74	0.47 ↑	2.01	0.0156	0.4695 NR_11001:LOC1019276	uncharacterized LOC101927630	chr7	-	17503069	17598533
17079293	5.67	4.62	0.75	0.29 ↑	2.07	0.0157	0.4695 NM_0577:CCNE2	cyclin E2	chr8	-	95891998	95908906
17044253	4.05	5.18	1.27	0.65 ↓	-2.2	0.0157	0.4695 NM_0010:GPNMB	glycoprotein (transmembrane) n	chr7	+	23275586	23314729
16733068	2.86	1.72	1.17	0.68 ↑	2.21	0.016	0.4734		chr11	+	1.25E+08	1.25E+08
16660038	3.26	5.08	1.03	0.9 ↓	-3.55	0.0168	0.4812 NM_0162:PADI3	peptidyl arginine deiminase, typ	chr1	+	17575593	17610728
16888510	1.09	2.34	0.67	1.03 ↓	-2.38	0.0185	0.4929		chr2	+	1.87E+08	1.87E+08
16693427	1.82	3.17	0.89	1.18 ↓	-2.55	0.0186	0.4929 NM_0029:S100A7	S100 calcium binding protein A7	chr1	+	1.53E+08	1.53E+08
16841634	2.31	3.41	0.96	0.55 ↓	-2.14	0.0193	0.4949 NM_0012:CDRT1	CMT1A duplicated region transcr	chr17	-	15468797	15523018
16831890	3.56	4.81	0.74	0.89 ↓	-2.37	0.0206	0.5014 NM_0010:LGALS9C	lectin, galactoside-binding, solub				

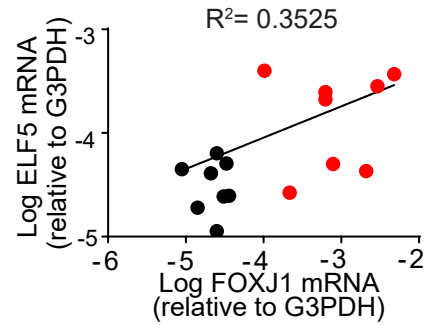
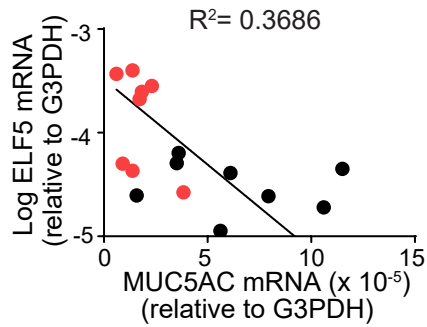
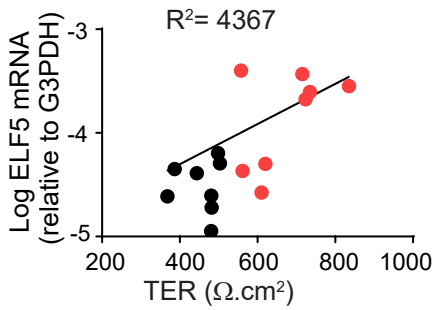
17125110	4.08	3	1.21	0.38	↑	2.12	0.0224	0.5127		TCONS_I2_+		1	381	
17000168	6.85	8.07	1.32	0.26	↓	-2.33	0.0228	0.5133	NM_004816:CXCL14	chemokine (C-X-C motif) ligand 1	chr5	-	1.35E+08	1.35E+08
16947041	2.47	1.27	1.28	0.69	↑	2.3	0.0228	0.5133	XR_92458:LOC105374111	uncharacterized LOC105374160	chr3	+	1.52E+08	1.52E+08
16936033	5.23	4.2	0.65	0.44	↑	2.04	0.024	0.5166			chr22	-	46020411	46020512
16844419	1.66	3.28	0.94	1.15	↓	-3.06	0.0241	0.5175	NM_0190:KRT24	keratin 24, type I	chr17	-	38854243	38860002
17012709	8.65	9.82	0.57	0.55	↓	-2.25	0.0262	0.5271	NR_00243:SNORD100	small nucleolar RNA, C/D box 10	chr6	+	1.33E+08	1.33E+08
16972291	1.96	3.04	1.13	1.12	↓	-2.11	0.0263	0.5271			chr4	+	1.7E+08	1.7E+08
16997010	1.75	0.65	1.09	0.62	↑	2.15	0.0327	0.5487	OTTHUMT:GTF2H2	general transcription factor IIH subunit	chr5	-	70328995	70330227
16761693	3.68	4.94	1.19	1.29	↓	-2.39	0.0351	0.5562	NR_03655:LINC01559	long intergenic non-protein coding RNA	chr12	-	13523605	13540101
17025738	0.94	2.02	0.66	2.29	↓	-2.11	0.036	0.5609	NR_11709:LOC441178	uncharacterized LOC441178	chr6	-	1.68E+08	1.68E+08
16804106	4.25	3.15	0.95	0.84	↑	2.14	0.0364	0.5629	NM_0013:ADAMTSL3	ADAMTS like 3	chr15	+	84322838	84708594
17126218	2.93	4.77	0.83	1.39	↓	-3.57	0.0372	0.5642			CDR1_AS	+	1	1487
17122142	3.67	5.05	1.75	1.24	↓	-2.59	0.0392	0.568			TCONS_I2_+		1	241
16990862	5.63	6.67	1.25	0.82	↓	-2.05	0.0406	0.5749	NM_0013:ABLIM3	actin binding LIM protein family	chr5	+	1.49E+08	1.49E+08
16851708	2.64	4.32	1.65	1.96	↓	-3.21	0.0411	0.5771	NM_0019:DSG1	desmoglein 1	chr18	+	28898052	28937394
17038059	2.96	1.38	1.23	1.19	↑	3	0.0431	0.5834			chr6_qbl_+		2661007	2677562
17121790	1.83	0.78	1.22	0.48	↑	2.08	0.0446	0.5871			TCONS_I2_+		1	2289
17125866	0.85	1.98	0.79	1.09	↓	-2.19	0.0471	0.596			gi3011733+		1	77
16829801	1.97	3	0.75	1.15	↓	-2.04	0.0474	0.5961	NM_0011:SPNS2	spinster homolog 2 (Drosophila)	chr17	+	4402129	4443228
17002898	6.63	5.19	1.25	0.56	↑	2.72	0.0488	0.5965	NM_0037:STC2	stanniocalcin 2	chr5	-	1.73E+08	1.73E+08

Supplemental Table 3. Biological Process terms enriched in COPD vs normal basal cells

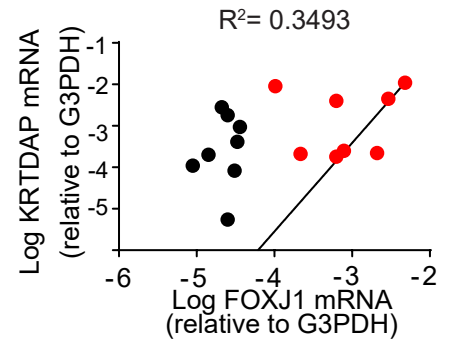
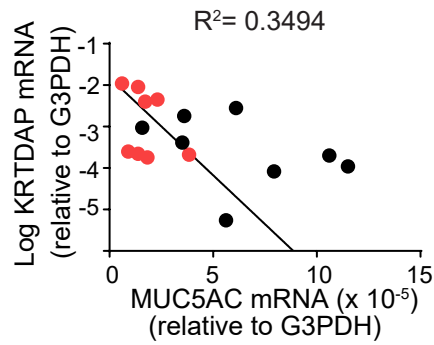
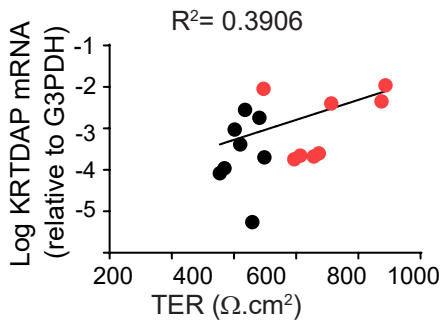
GeneSet	description	size	overlap	expect	enrichmentRatio	FDR p-value
GO:0008544	epidermis development	437	11	1.2	8.8	2.5E-04
GO:0019730	antimicrobial humoral response	106	6	0.3	19.9	1.6E-03
GO:0070268	cornification	106	6	0.3	19.9	1.6E-03
GO:0030216	keratinocyte differentiation	280	8	0.8	10.0	1.9E-03
GO:0061844	antimicrobial humoral immune response mediated by antimicrobial peptide	65	5	0.2	27.0	1.9E-03
GO:0043588	skin development	390	9	1.1	8.1	1.9E-03
GO:0031424	keratinization	210	7	0.6	11.7	2.5E-03
GO:0009913	epidermal cell differentiation	331	8	0.9	8.5	3.9E-03
GO:0006959	humoral immune response	233	7	0.7	10.5	3.9E-03
GO:0030855	epithelial cell differentiation	712	10	2.0	4.9	2.1E-02
GO:0009888	tissue development	1839	16	5.2	3.1	2.1E-02
GO:0002325	natural killer cell differentiation involved in immune response	5	2	0	140.4	4.7E-02
GO:0032826	regulation of natural killer cell differentiation involved in immune response	5	2	0	140.4	4.7E-02
GO:0018101	protein citrullination	5	2	0	140.4	4.7E-02
GO:0036414	histone citrullination	5	2	0	140.4	4.7E-02

Supplemental Figure 1

A



B



C

