SUPPLEMENTARY INFORMATION

Title

Osteopontin expression in small airway epithelium in COPD is dependent on differentiation and confined to subsets of cells

Authors

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Figure S1. Phenotypic characterization of OPN-expressing cells in small airways of a never smoking controls

Immunofluorescence was used to detect possible co-localization of OPN and mucin 5AC (MUC5AC) in goblet cells **(A)**, uteroglobin (UTG) in club cells **(B),** p63 in basal cells **(C)**, and forkhead box protein J1 (FOXJ1) in ciliated cells **(D)**. In the overlay, a high degree of co-localization was observed for OPN and MUC5AC in goblet cells and UTG in club cells. One representative donor out of three. Scale bars = 100 µm.

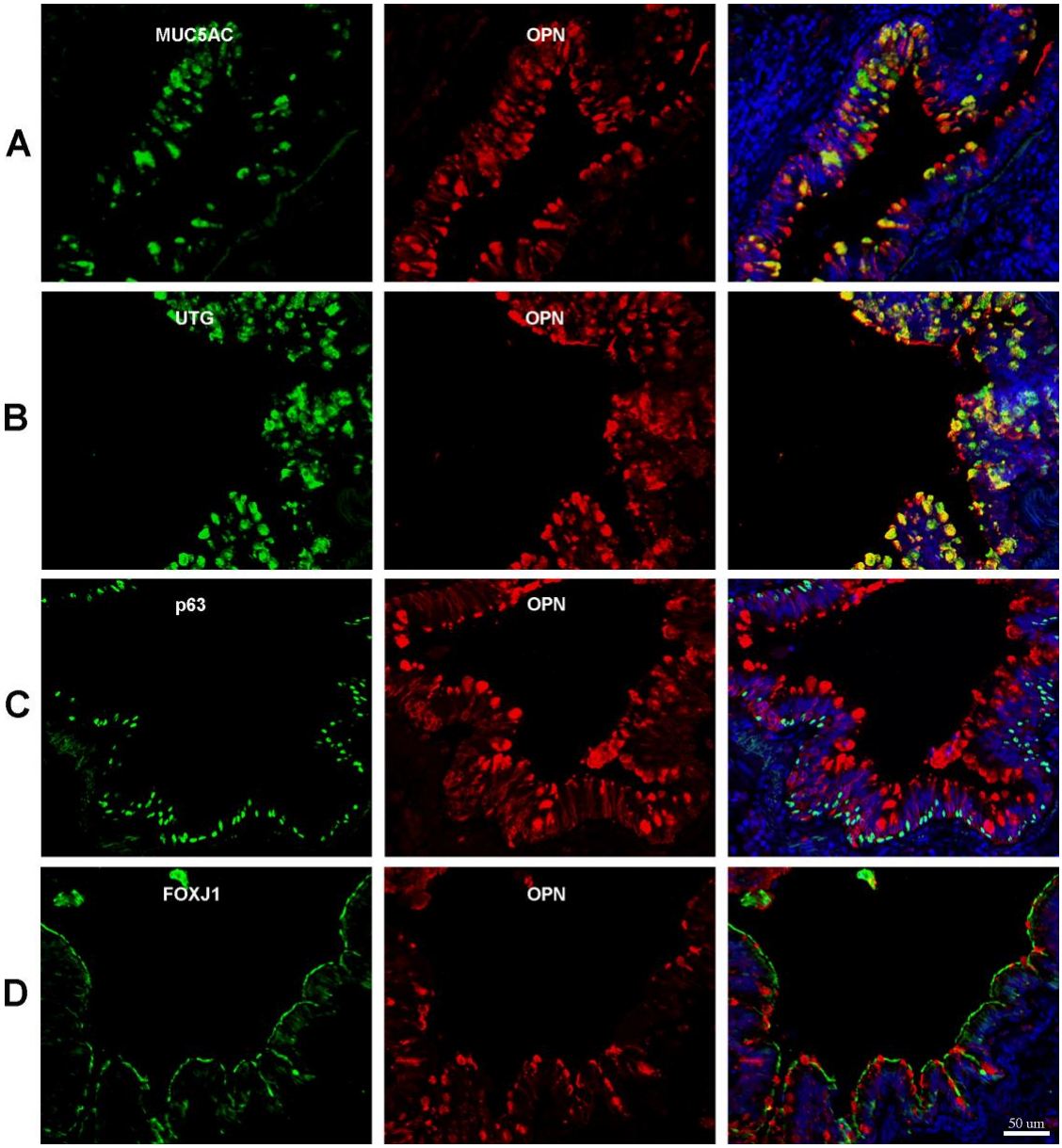

Table S1. Subject characteristics

Characteristic	Never-smokers	Smokers w/o COPD	GOLD I COPD	GOLD II-III* COPD	GOLD IV COPD
Subjects, n	8	6	6	12	9
Gender, male/female	2/6	2/4	4/2	9/3	4/5
Age, years	66 (33-76)#	57 (47-68)	68 (56-75)	66 (53-77)	62 (53-66)
Smoking history, pack-years	0	36 (20-80)	39 (25-66)	46 (30-65)	40 (25-60)
Smoking status, ex-smokers/current	NA	2/4	3/3	7/5	10/0
FEV_1	2.5 (1.7-5.1)	2.8 (1.9-3.5)	2.9 (1.6-3.2)	1.8 (1.2-2.9)	0.6 (0.4-1.0) □ □
FEV_1, % of predicted	109 (82-141)	95 (82-120)	86 (80-95)	67 (43-78)	24 (15-27) □ □ □
FEV_1/(F)VC, %	82 (66-121)	76 (71-88)	67 (65-70)	61 (41-68)	32 (20-39)
Inhaled β_2-agonists					
Short-acting (yes/no/unknown)	0/8/0	0/6/0	1/5/0	2/10/0	4/4/1‡
Long-acting (yes/no/unknown)	0/8/0	0/6/0	0/6/0	0/12/0	3/5/1‡
Inhaled anticholinergics					
Short-acting (yes/no/unknown)	0/8/0	0/6/0	1/5/0	2/10/0	2/6/1‡
Long-acting (yes/no/unknown)	0/8/0	0/6/0	0/6/0	0/12/0	5/3/1‡
Inhaled short-acting β_2-agonist plus anticholinergics (yes/no/unknown)	0/8/0	0/6/0	0/6/0	0/12/0	3/5/1‡
Corticosteroids					
Inhaled (yes/no/unknown)	0/8/0	0/6/0	0/6/0	1/11/0	2/6/1‡
Oral (yes/no/unknown)	0/8/0	0/6/0	0/6/0	0/12/0	2/6/1‡
Inhaled long-acting β_2-agonist plus corticosteroids (yes/no/unknown)	0/8/0	0/6/0	0/6/0	1/11/0	6/2/1‡
Mucolytics (yes/no/unknown)	0/8/0	0/6/0	2/4/0	0/12/0	5/3/1‡

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; FEV_1 = forced expiratory volume in one second; (F)VC = (forced) vital capacity; GOLD = Global Initiative for Chronic Obstructive Lung Disease.

Values are median (range) or n.

* Two patients with GOLD Stage III COPD (median value of FEV_1% of predicted, 44.5%; range 43.2-45.9).

The mean value of the study group is 63 years.

‡ One patient with unknown medical history.

Table S2. Primer sequences

Gene	Forward Primer	Reverse Primer
OPN	5'-GGACTCCATTGACTCGAACGACTCTG-3’	5'-AACCACACTATCACCTCGGCCATC-3’
FOXJ1	5'-GGAGGGGACGTAAATCCCTA-3’	5'-TTGGTCCCAGTAGTTCCAGC-3’
MUC5AC	5'-ATTTTTTCCCCACTCCTGATG-3’	5'-AAGACAACCCACTCCCAACC-3’
p63	5'-CCACCTGGACGTATTCCACTG-3’	5'-TCGAATCAAATGACTAGGAGGGG-3’
UTG	5’-CCCTGGTCACACTGGCTCTCTGC-’3	5’-CCCCTGCCTCCCTCATGTCTTG-’3
RPL13A	5'-AAGGTGGTGGTCGTACGCTGTG -3’	5'-CGGGAAGGGTTGGTGTTCATCC-3’

OPN, osteopontin; FOXJ1, forkhead box protein J1; MUC5AC, mucin 5AC; p63, tumor protein p63; UTG, uteroglobin; RPL13A, ribosomal protein L13A.