Distinct Roles of FOXA2 and FOXA3 in Allergic Airway Disease and Asthma

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Online Data Supplement

Supplementary Methods

FOXA2 staining in biopsies from human subjects

We studied sections of formalin fixed paraffin embedded endobronchial biopsies stored in the Airway Tissue Bank at the University of California, San Francisco (UCSF). The tissue bank is approved by UCSF's Committee on Human Research and administered by Drs. Prescott Woodruff and John Fahy. The endobronchial biopsies had been collected during research bronchoscopy from 5 non-smoking subjects with asthma and 5 healthy non-smoking controls (Table E1). Asthmatic subjects had a prior physician diagnosis of asthma, PC20 methacholine <8 mg/mL, and were using only inhaled beta-agonist medications for therapy. Healthy controls were non-smokers with no history of lung disease and PC20 methacholine >16 mg/mL. Spirometry, methacholine challenge, and bronchoscopy had been performed using methods described previously (E1). At bronchoscopy, 4-6 endobronchial biopsies were taken from 2nd through 4th order carinae of lower lobe, middle lobe, and upper lobe segments and embedded together in a single paraffin block using methods that ensured isotropic uniform random orientation (E2). Sections were stained using FOXA2 antiserum and with 4',6-diamidino-2-phenylindole to identify nuclei. All areas with airway epithelial cells were photographed and nuclei with mean pixel intensity values exceeding a pre-determined threshold were characterized as FOXA2-positive by a blinded observer. The ratio of FOXA2-negative nuclei to FOXA2-positive nuclei was determined for each subject. Design-based stereology was applied to measure stored mucin volume in tissue sections from the same biopsies.

References

E1. Innes, A. L., P. G. Woodruff, R. E. Ferrando, S. Donnelly, G. M. Dolganov, S. C. Lazarus, and J. V. Fahy. 2006. Epithelial mucin stores are increased in the large airways of smokers with airflow obstruction. *Chest* 130(4):1102-8.

E2. Ferrando, R. E., J. R. Nyengaard, S. R. Hays, J. V. Fahy, and P. G. Woodruff. 2003. Applying stereology to measure thickness of the basement membrane zone in bronchial biopsy specimens. *J Allergy Clin Immunol* 112(6):1243-5.

Figure E1: Allergen challenged *Foxa3*$^{-/-}$ mice have increased IgE production and eosinophilic inflammation but reduced airway reactivity. (A) Serum ovalbumin (OVA)-specific IgE was measured in saline- and ovalbumin-challenged control and *Foxa3*$^{-/-}$ mice. Airway inflammation was analyzed by counting macrophages (Mac), eosinophils (Eos), lymphocytes (Lymph), and neutrophils (Neut) in bronchoalveolar lavage fluid. (C) Airway reactivity to intravenously administered acetylcholine was measured using the Flexivent system. Results are means ± s.e.m for 12-20 mice per group. * $P < 0.05$, ** $P < 0.01$ compared to Saline-challenged mice. † $P < 0.05$ compared to ovalbumin-challenged *Foxa3*$^{-/-}$ mice.

Table E1: Subject characteristics

	Control (n=5)	Asthmatics§ (n=5)
Age (years)	34.0 ± 9.0	37.0 ± 9.9
FEV1* (% predicted)	107.0 ± 13.5	92.4 ± 16.3
Methacholine PC_{20}† (mg/ml)	> 16	< 4
‡Skin test reactivity	4/5	5/5

* Forced expiratory volume in 1 s; 4/5 asthmatics had mild disease (FEV1 > 80% predicted), 1/5 had moderate disease (FEV1 = 60-80% predicted).

† Provocative concentration of methacholine that caused a 20% reduction in FEV1.

‡ Positive for one or more skin prick tests.

§ All asthmatics were using short acting beta-agonists but none used other medications.

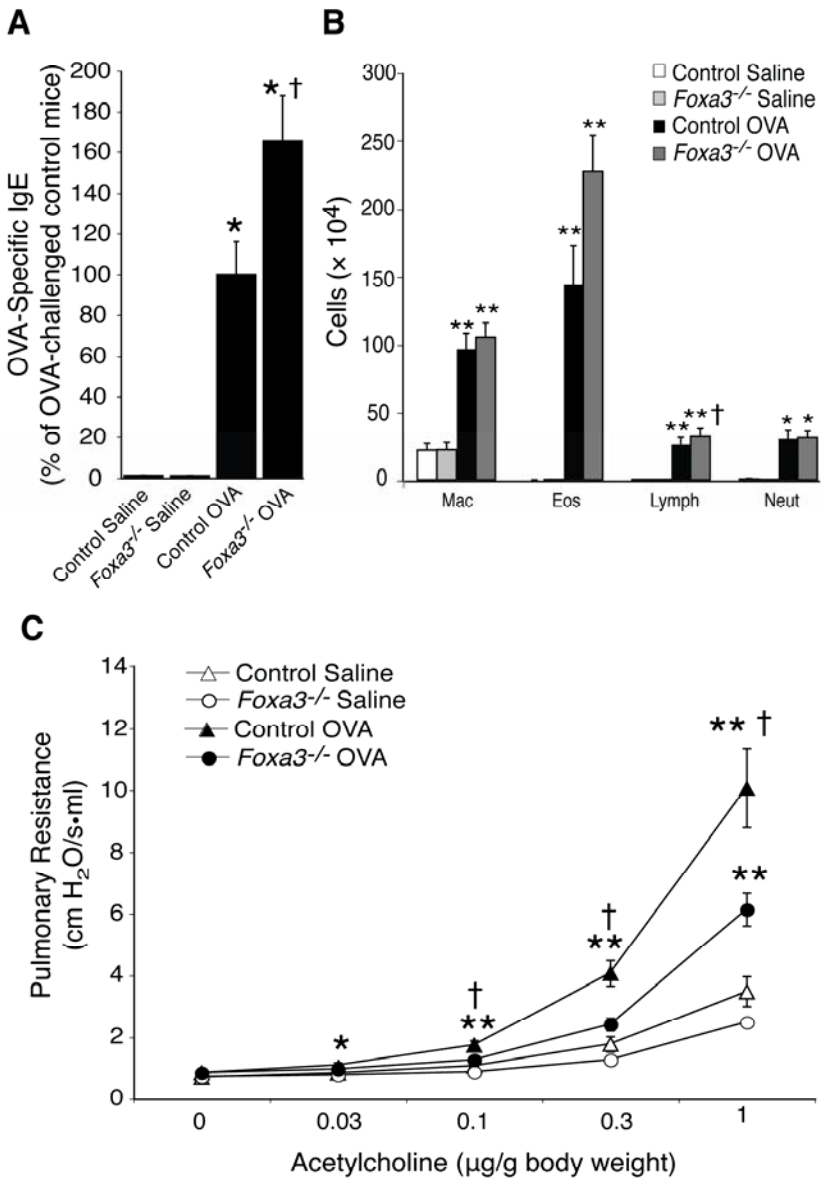

Figure E1, Park