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Genetic Variation in Surfactant Protein-A2 Results in Altered Regulation of Eosinophil Activities and Enhanced Eosinophilia in Patients with Asthma

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Humans express a repertoire of single amino acid genetic variant proteins for surfactant protein (SP)-A1 and SP-A2 that may be associated with certain lung physiology and disease. We have previously shown that SP-A mediates eosinophil degranulation and that certain allelic variants of SP-A2 are dysfunctional in asthma. We therefore hypothesized that variation in SP-A2 at position Gln(Q)223Lys(K) leads to altered regulation of eosinophil activities and that patients with asthma who harbor the minor allele may demonstrate enhanced eosinophilia. SP-A was extracted from the lavage of patients with alveolar proteinosis and genotyped for the SP-A2 223Q/K locus. Genotyped SP-A was incubated with eosinophils *in vitro* to assess regulation of eosinophil activity. Humanized SP-A transgenic mice were created that represent single allelic variant changes in SP-A2 protein, designated SP-A2 223Q and SP-A2 223K. Humanized mice were challenged in an allergic model to determine the effect of SP-A genetic variation during allergic inflammation. We discovered that SP-A2 223Q inhibits eosinophil degranulation and leads to reduced viability of eosinophils in culture conditions. In contrast, SP-A2 223K is unable to attenuate eosinophil degranulation and has no effect on eosinophil viability. Humanized SP-A2 223Q mice have significantly reduced mucus production in an allergic model compared with SP-A$^{-/-}$ mice, whereas mice expressing SP-A2 223K were not different from mice that are devoid of SP-A. Additionally, from genotyped samples obtained from subjects with mild to moderate asthma, we discovered that subjects with asthma harboring one or more copies of the minor allele (223K) had a greater percentage of eosinophils in their bronchoalveolar lavage and serum. These studies suggest that genetic variation in SP-A2 at position SP-A2 Gln223Lys is an important mediator of eosinophil activities, which may lead to a more severe asthma phenotype.

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Antisense MicroRNA Therapy of Airway Remodeling in House Dust Mite–sensitized Mice

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There is compelling need for mechanistically novel antiinflammatory and antiremodeling drugs for therapy of severe asthma. Our goal is to develop systemically administered agents that provide long-acting suppression and reversal of lung inflammation, mucosal metaplasia, and airway structural cell remodeling. To inhibit remodeling, microRNA (miR)-145 was targeted because it is a key regulator of structural cell differentiation and inflammation. An antisense, locked nucleic acid/DNA oligonucleotide complementary to nucleotides 2–16 of hsa-miR-145–5p (anti–miR-145) was delivered to the lungs via a novel lipid nanoparticle administered intravenously. After sensitization with house dust mite (HDM), 2 cohorts of 10 mice were treated with dextrose or anti–miR-145 nanoparticles (2 mg/kg, intravenously, on Days 13, 15, and 17). A third cohort of control mice was not sensitized to HDM and was not treated with anti–miR-145. Bronchoalveolar lavage (BAL) was performed on Day 18, and lungs were fixed with formalin. There was significant eosinophilia of the BAL fluid after challenge with HDM, and increased CD68 immunostaining of tissue sections, which verified the effectiveness of HDM challenge. Airway and vascular wall remodeling and increased mucin-producing cells were observed in HDM-challenged animals. Mice treated with anti–miR-145 showed reduced BAL eosinophilia, reduced obstructive airway remodeling, reduced mucosal metaplasia, and reduced CD68 immunoreactivity. Anti–miR-145 delivered intravenously distributed to most cells in the lung parenchyma, as shown by *in situ* hybridization. Antagonizing the function of miR-145 in the lung significantly reduces obstructive remodeling in a short-term HDM mouse model of asthma. These results also establish the biodistribution and efficacy of anti–miR-145 delivered via the blood compartment, which bypasses obstructed airways that can limit distribution and efficacy of inhaled antisense oligonucleotides.

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Compressive Stress Causes an Unjamming Transition and an Epithelial–Mesenchymal Transition in the Airway Epithelium in Asthma

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One of the major characteristics of asthma is bronchospasm, which is caused by excessively contracted smooth muscle and results in buckling of the airway epithelium. Buckling of the epithelium imposes compressive mechanical stress on the airway epithelial cells. We previously reported that compressive mechanical stress, which is similar to stress that occurs during bronchospasm, induces airway remodeling in well-differentiated primary human bronchial epithelial (HBE) cells maintained in air–liquid interface culture. In this study, we investigated the physical behavior of HBE cells cultured from normal donors and donors with asthma to gain a better understanding of the mechanobiology of airway epithelial cells.

We captured time-lapse images of HBE cells and quantified cellular motions. In normal cells, the transition from an unjammed state, in which cells flow like a fluid and rearrange with neighbors frequently, to a jammed state, in which cells move little, like a solid, and rearrange infrequently, occurred between Days 6 and 8 in air–liquid interface culture, but in asthmatic cells the transition was delayed until Day 14 (1). Also, in cell lysates from Days 6 through 14, the existence of vimentin was temporally correlated with the onset of the jamming transition. Treatment of asthmatic cells with SB431542, a transforming growth factor-β receptor inhibitor, accelerated the jamming transition and the disappearance of vimentin. Furthermore, compressive stress caused the jammed cells to transition to an unjammed state, but pretreatment with SB431542 attenuated the compressive stress-induced unjamming transition. Repeated applications of compressive stress induced the expression of vimentin and slug (epithelial–mesenchymal transition marker proteins) but reduced claudin-1 (a tight junction protein).

Maturation of unjammed HBE cells causes a jamming transition, whereas compression of the jammed HBE cells causes an unjamming transition. Importantly, transforming growth factor-β controls the jamming transition and associated vimentin expression, and compressive stress induces EMT. These findings suggest that the jamming–unjamming transition is a novel physical feature that captures injury-repair processes and differentiates the normal epithelium from the asthmatic epithelium.

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Longitudinally Stable, Clinically Defined Clusters of Patients with Asthma Independently Identified in the ADEPT and U-BIOPRED Asthma Studies

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Background: ADEPT (Airways Disease Endotyping for Personalized Therapeutics) and U-BIOPRED (Unbiased Biomarkers for the Prediction of Respiratory Disease Outcome Consortium) are independent asthma biomarker studies that aim to enable personalization of therapies.

Methods: Patients in both studies were identified by similar criteria, and similar clinical parameters and biomarkers were assessed in blood, sputum, and airway samples. Fuzzy partition-around-medoid clustering was performed on the ADEPT dataset (n = 154) and independently on the U-BIOPRED asthma dataset (n = 82), filtered to match ADEPT inclusion criteria. For both studies, the same eight easily measurable clinical variables were used, and ADEPT also included methacholine airway hyperresponsiveness. Models for cluster classification probabilities were derived and applied to the 12-month longitudinal ADEPT data and the full U-BIOPRED adult asthma dataset (n = 397) as independent external validation.

Measurements and Main Results: Four clusters were identified in the ADEPT-asthma study population with distinct clinical