Whole genome DNA methylation marks in bronchial epithelium associated with *Aspergillus fumigatus* exposure

ISSUE/PURPOSE

Airborne exposures to fungi (molds) are common in agricultural settings. *Aspergillus fumigatus*, is a fungi whose spores are frequently detected in the agricultural environment. Diseases such as farmer's lung, asthma, hypersensitivity pneumonitis and cancer have been associated with various *Aspergillus* species exposures, yet, there are no federally accepted health-based standards for safe mold levels. Upon inhalation, normal human bronchial epithelial (NHBEs) cells are the first cells to come in contact with the inhaled *A. fumigatus* spores. These cells not only play an important role in providing defense and inflammation against fungal spores, but can also orchestrate the subsequent lung repair (fibrotic) response to maintain homeostasis. The **purpose** of this pilot project was to answer a key, yet unanswered question: what is the impact of environment (*A. fumigatus* exposure) in modulating molecular events in NHBEs.

APPROACH OR PROJECT MILESTONES

Our central hypothesis for this project was that exposure to *A. fumigatus* will lead to a temporally distinct molecular signature, specifically epigenetic (whole genome DNA methylation) events in epithelial cells that would contribute to its function in context of an airway disease. To test our hypothesis we proposed the following specific aim: Determine an extent to which *A. fumigatus* exposure induces whole genome DNA methylation events in the NHBEs. Towards this specific aim, we incubated the NHBEs with *A. fumigatus* spores for a predetermined time (early timepoint), in the presence of low dose antifungal cocktail. At the end of co-incubation period, unbound conidia were removed by washing. At this point, either the NHBEs were collected for DNA and RNA extraction or were cultured for additional time (later timepoint) in a high dose anti-fungal containing media and then collected for DNA and RNA extraction. Whole genome DNA methylation analysis of NHBEs, with or without exposure to *A. fumigatus* exposure, was performed using Illumina Infinium Human Methylation450 bead array technology. The bioinformatic/biostatistical data analysis is being performed using RnBeads R statistical software.

KEY FINDINGS/RESULTS

The Illumina assay was a success for all the samples. Preliminary analysis shows differentially methylated genes, promoters and CpG islands in *A. fumigatus* treated epithelial cells as compared with the control samples. Temporal differences have also been observed in methylation of genes, promoters and CpG islands upon comparison of *A. fumigatus* treated epithelial cells at early and late timepoints. However, an in-depth analysis is currently underway and will be reported in the next report, due at the end of August 2016.

THE BOTTOMLINE

If successful, the immediate implication of this project would be to design-evidence based therapeutic and diagnostic standards for mold-associated diseases, airway remodeling, allergy, cancer and exposures in agricultural settings, respectively. Once established, this model is expected to also be useful for testing other airborne agricultural exposures, so we expect the results of this study to be very valuable in terms of securing the necessary preliminary data to compete for other federal funding and establish a long term research program of interest to the agricultural community.