Asthma is an inflammatory disease associated with chronic perturbation of homeostatic mechanisms, leading to alterations in the structure of the airway walls, termed airway remodeling (AR). Lung fibrosis is characterized by increased deposition of extracellular matrix (ECM) proteins, especially collagens, and enhanced proliferation and activation of fibroblasts, and, ultimately, distortion of normal lung architecture and loss of respiratory function. Arginase is a key enzymatic site of nitric oxide and polyamine synthesis. Its increased activity and expression, especially in asthma, are associated with enhanced deposition of extracellular matrix (ECM) proteins, cell hyperplasia, and increased airway smooth muscle mass, subepithelial fibrosis, epithelial changes, and increased numbers of fibroblasts and epithelial cells [1]. In addition, AR is resistant to current medical therapies and can lead to irreversible airflow limitation, thereby increasing the severity of asthma [1].

Hypothesis: We hypothesized that L-arginine metabolism is altered in asthma, and may contribute to airway remodeling mechanisms, likely by upregulation of expression of genes involved in arginine and polyamine metabolism.

Methods: We investigated expression of key regulatory enzymes in human nasal polyps, human lung fibroblasts, and normal lung fibroblasts (NHLF) by Western blot and cell immunostaining in human lung fibroblast cell lines. All samples were obtained from Lonza. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum. Following 24h incubation at 37°C, cells were washed with ice-cold DMEM (no serum). Cells were then incubated with 20% DMSO for 48h at 37°C. Samples were loaded onto 15% Tris-glycine polyacrylamide gels, transferred to nitrocellulose membranes via the Invitrogen iBlot system. Membranes were blocked overnight at 4°C in Pierce Fast Western antibody diluent containing 3% non-fat milk or 3% bovine serum albumin for Arginase I, Arginase II, Ornithine Decarboxylase (ODC), Ornithine Aminotransferase (OAT), 8-Tubulin, and Cyclin A (abcam). All antibodies were supplied by Santa Cruz Biotechnology. Blots were washed in PBS-Tween-20 (0.1%) and then incubated for 1h at RT in antibodies diluting containing Fast Western Optimized HRP Reagent and BIO-RAD Precision Protein StrepTactin-HRP Conjugate. Blots were again washed using PBS-Tween buffer. Protein bands of interest were visualized using Clarity ELL Western Substrate (Bio-RAD).

Results: Arginase I, arginase II, ODC, and OAT are present in NHLF and DHLF cells, with or without 360 nm, 488 nm, and 554 nm wavelengths to view nuclei, mitochondria, and endoplasmic reticulum, respectively. Western blots were stained with specific primary antibodies (anti-Arginase I, anti-Arginase II, anti-ODC, and anti-OAT) and were the same antibodies used for Western blot. Membranes were loaded onto 4-15% gradient Mini-PROTEAN TGX Gels. Bio-RAD Precision Protein Standards were run on each gel as a molecular weight marker. Membranes were probed with horseradish peroxidase (HRP)–conjugated secondary antibodies and developed using chemiluminescent ECL Western Substrate (Bio-RAD). Levels of Arginase I, Arginase II, Ornithine Decarboxylase, Ornithine Aminotransferase, 8-Tubulin, and Cyclin A were detected in NHLF and DHLF, respectively. Positive signals for the proteins of interest were observed. Membranes were stained with specific primary antibodies (anti-Arginase I, anti-Arginase II, anti-ODC, and anti-OAT) and were the same antibodies used for Western blot. Membranes were loaded onto 4-15% gradient Mini-PROTEAN TGX Gels. Bio-RAD Precision Protein Standards were run on each gel as a molecular weight marker. Membranes were probed with horseradish peroxidase (HRP)–conjugated secondary antibodies and developed using chemiluminescent ECL Western Substrate (Bio-RAD). Levels of Arginase I, Arginase II, Ornithine Decarboxylase, Ornithine Aminotransferase, 8-Tubulin, and Cyclin A were detected in NHLF and DHLF, respectively. Positive signals for the proteins of interest were observed.

Conclusions: In both normal and diseased human lung fibroblasts with or without 360 nm, Arginase I, arginase II, ODC, and OAT are present in cell immunostaining. The Western blot technique was able to show localization of mitochondrial and localization of proteins of interest. This study provides a powerful tool for the study of arginine metabolism in asthma and may have clinical relevance in asthma. Further research is needed to determine the role of arginine metabolism in asthma and its potential as a therapeutic target.

REFERENCES