Characterization of the Function of Carbonic Anhydrase 8

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Background/Objective: Severe asthma is a complex pulmonary disease characterized by airway inflammation, bronchoconstriction, and acid-base dysregulation. In the Severe Asthma Research Program bronchoscopies, transcriptomics showed CA8 as a gene that is strongly associated with asthma severity. CA8, however, lacks classical CA enzyme function: it does not catalyze hydration and dehydration of CO2. The function of CA8 in the airway epithelium remains unknown. We hypothesize that CA8 serves a protective role in the airway due to its downregulation in patients with severe asthma. We aim to characterize the function of CA8 by studying its potential as an enzymatic protein.

Methods: We used colorimetric assays to detect and quantify nitrogen oxides. We tested for S-nitrosothiol synthase, denitrosylase, nitrate and nitrite synthase, and nitrite reductase activities using the Griess reagent in conjunction with Saville denitrosylation reagents and with reduction using vanadium chloride. Samples were incubated for 60 minutes. We then went on to design a metabolomic experiment in which products will be identified by NMR: for these, we transfected Chinese hamster ovary (CHO) cells using lentivirus containing GFP-labeled CA8 or empty vector (negative control).

Results: CA8 protein does not have these following enzymatic functions: SNO synthase, denitrosylase, nitrite and nitrate synthase, and nitrite reductase. We successfully transfected with GFP-labeled CA8 and are awaiting results of the metabolomic studies.

Conclusion and Potential Impact: Isolated CA8 does not appear to have any nitrogen oxide redox activities relevant to asthma**.** The next steps include confirmatory western and SNO western blots to determine protein s-nitrosylation using transfected CHO whole cell lysate. Extracellular medium pH will also be measured. We will then move on to NMR-based metabolomics. This will help us better understand the biochemical mechanisms of CA8. Ultimately, this can provide researchers with a novel approach to asthma treatments.