Regulation of Human Ileal Biopsy and Intestinal Organoid ROS Production and Gene Expression by *DUOX2* Genetic Variation and Microbial Metabolites

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Introduction: The *DUOX2* intestinal epithelial NADPH oxidase is upregulated in Crohn's Disease (CD) by microbial products, and *DUOX2* loss-of-function mutations are associated with increased CD risk. The microbial metabolite butyrate enhances intestinal mitochondrial and barrier function and reduces inflammatory signals driving fibrosis.

Hypothesis: We hypothesized that *DUOX2* genetic loss-of-function, and exposure to the microbial metabolite butyrate, would be associated with variation in cellular reactive oxygen species (ROS) production, and mitochondrial and extra-cellular matrix (ECM) gene expression regulating the balance between wound healing and tissue fibrosis.

Methods: RNASeq gene expression data from CD and non-IBD control ileal biopsies was tested for differences in mitochondrial gene expression between CD patients with inflammatory (B1) versus fibrotic stricturing (B2) behavior, and wild type (WT) and *DUOX2* variant genotypes. ROS production was measured by flow cytometry, and mitochondrial and ECM gene expression was measured by RT-PCR, in EpCAM+ epithelial cells and CD90+ fibroblasts from WT and *DUOX2* variant human intestinal organoids (HIOs) following butyrate exposure.

Results: 34 mitochondrial genes, notably those of the *ATP*, *COX*, and *NDUF* families which encode for respiratory chain complexes, were significantly downregulated ($p<0.05$) in B2 patients who ultimately developed strictures compared to their B1 counterparts. The notable exception was with *HIF1A*, which was found to be upregulated ($p<0.01$) in B2 patients likely as a result of increased oxidative stress. Analysis of mitochondrial gene signatures did not yield any significant differences when WT was compared against those that had 1 or 2 *DUOX2* variants. However, increased mitochondrial gene expression ($p<0.05$) was observed in patient samples carrying 3 or 4 *DUOX2* variants. Butyrate exposure prevented pyocyanin-induced ROS production in WT HIO EpCAM+ and CD90+ cells, but this effect was abolished in *DUOX2* variant HIO cells. WT HIO EpCAM- stromal cells demonstrated upregulation of four mitochondrial genes (*COX5B, NDUFA1, POLG2, SLC25A27*) and suppression of two collagen genes (*COL1A1 and COL4A5*) by butyrate ($p<0.05$).

Conclusions: A protective ileal mitochondrial gene signature is preserved in CD patients carrying *DUOX2* mutations. Butyrate reduces HIO ROS production in a DUOX2-dependent manner, and regulates stromal cell mitochondrial and ECM gene expression, supporting further development of microbial therapies in CD.

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