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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