Identifying Epithelial Inflammatory Signals Driving the Proliferation of Myofibroblasts in DUOX2 Genetic Variant Human Intestinal Organoids

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Introduction: Up to 15% of children with Crohn's Disease (CD) undergo bowel resections for strictures within three years of diagnosis. Patients who develop strictures exhibit reduced expression of mitochondrial (MITO) genes, and increased expression of inflammatory (INFL) and extra-cellular matrix (ECM) genes, at diagnosis. Mutations in *DUOX2*, a gene encoding the NADPH oxidase complex in intestinal epithelial cells (IEC), are associated with risk for CD, and development of strictures. We reported that IL1B expressing macrophages are increased in patients with refractory disease, while the cyclooxygenase and lipoxygenase inhibitor ETYA was predicted to inhibit inflammatory signals and promote effective wound healing. Their effects in the human intestinal organoid (HIO) model system were not known.

Hypothesis: We hypothesized that IL1B and ETYA would regulate pathways implicated in CD strictures in the HIO model system, and that this would vary with *DUOX2* genotype.

Methods: HIO were derived from wild type (WT) and *DUOX2* mutant (DUOX2^{var}) induced pluripotent stem cells (iPSCs) prepared from CD patients. HIO tissue stiffness was measured using atomic force microscopy (AFM). HIO were exposed to IL1B for 24 hours and ETYA for 3 days, and RNA was prepared and expression of MITO, INFL, and ECM genes was determined using a TaqMan Low Density Array (TLDA) card. A Luminex panel including 14 cytokines and growth factors prioritized by patient-based ileal transcriptomic analysis was used to test variation in secreted cytokines and growth factors. ROS production in EPCAM+ epithelial cells and CD90+ stromal cells was measured by flow cytometry.

Results: WT HIO exhibited tissue stiffness comparable to normal human ileum; this was significantly reduced in DUOX2^{var} HIO. IL1B upregulated secretion of the INFL proteins CXCL1 and MCP-3 in both WT and DUOX2^{var} HIO, and up-regulated CXCL5 and CXCL6 only in WT organoids. ETYA up-regulated expression of the *COX5B*, *HIF1A*, *POLG2*, and *SLC25A27* MITO genes associated with lower rates of strictures, while reducing expression of the *ACTA2*, *VIM*, and *COL1A1* ECM genes associated with higher rates of strictures, independent of *DUOX2* genotype. Conversely, ETYA suppressed production of the INFL proteins CXCL5, CXCL6, CXCR1, and MCP-3 only in WT HIO; no effect was observed in DUOX2^{var} HIO. Consistent with this, ETYA reduced superoxide production by HIO EPCAM+ epithelial cells only in WT organoids; no effect was observed in DUOX2^{var} HIO. This was specific, as no effect of ETYA upon ROS production was detected in CD90+ stromal cells.

Conclusions: Our data regarding tissue stiffness confirmed that HIO provide a relevant model system to study genetic and environmental mechanisms regulating wound healing and strictures in CD. ETYA regulated MITO and ECM genes independent of *DUOX2* genotype, and thus may have a broad therapeutic effect in preventing strictures. Conversely, effects of ETYA upon ROS production and INFL protein secretion were observed only in WT HIO, suggesting that *DUOX2* mutations alter key ETYA dependent anti-inflammatory signaling pathways. Ongoing studies will therefore test effects of ETYA ± IL1B upon HIO mitochondrial function, ROS production, and tissue stiffness, in the context of *DUOX2* genotype.

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