Evaluating Cytokine and Pro-Fibrotic Extracellular Signaling in DUOX2 Genetic Variant Human Intestinal Organoids with Microbiota Metabolite Exposures

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Introduction. Crohn's Disease (CD) is a chronic inflammatory illness affecting the gastrointestinal tract [1] which can progress to stricturing fibrotic complications requiring surgery [2,3,4]. Half of adult patients develop strictures within 20 years of diagnosis [5,6]. CD is currently treated with anti-inflammatory medications, with biologics targeting inflammatory cytokines having the greatest efficacy [7]. However, a considerable number of patients do not achieve a sustained clinical remission[7,8,9]. There are currently no approaches that specifically address fibrosis [7], with stricturing an exclusion criterion for most clinical trials [10–19]. This project will focus on understanding pathogenic mechanisms underlying the structuring phenotype, and elucidating potential targets for personalized anti-fibrotic therapeutics.

To test interactions between host genetics (DUOX2) and bile acids (chenodeoxycholic acid and lithocholic acid), we have developed a novel induced pluripotent stem cell (IPSC) derived macrophage:human intestinal organoid (mac:HIO) co-culture system. The objective of this project was to utilize our novel mac:HIO model system with exposures to bile acids to test mechanisms driving macrophage-dependent HIO collagen production.

Methods. iPSC was differentiated and HIOs generated[10]. We used the STEMdiff™ Hematopoietic Kit to generate hematopoietic progenitor cells, which were differentiated into macrophage-like cells using rhGM-CSF and rhM-CSF. Macrophages were characterized by flow cytometry with CD14, CD64, CD68, and CD163 staining, and by cytospin for morphological analysis. We studied mac:HIO co-cultures under basal conditions, and following LPS (100 ng/mL) +/- CDCA or LCA exposure (50 μM)[11]. We anticipated that two weeks was the ideal exposure time to regulate HIO collagen content, while shorter exposures of 24-72 hours helped to define effects upon cytokine production. We tested cytokine, chemokine, and growth factor production using a custom Luminex assay, and expression of pro-fibrotic ECM genes using a custom PCR array. Immunofluorescence with DAPI co-staining included e-cadherin (CDH1) for epithelial cells, alpha-smooth muscle actin ($ACTA2^+$) and vimentin (VIM^+) for myofibroblasts, and $ACTA2VIM^+$ for fibroblasts. Sirius red immunohistochemistry (Abcam) with polarized light microscopy helped to define collagen content quantified.

Anticipated results. We **anticipate** that, relative to HIO and macrophage-like cells with the DUOX2 reference genotype, $DUOX2^{var}$ mac:HIO will exhibit <u>increased</u> HIO collagen content in the LPS primed mac:HIO system that has CDCA exposure, in conjunction with increased proinflammatory cytokine production, and increased ECM gene expression. Conversely, LCA exposure will lead to reduced cytokine production and prevent increases in HIO ECM gene expression and collagen content, <u>independent</u> of DUOX2 genotype.

Conclusion. These data will provide a mechanism which will begin to explain the increase in stricturing observed in CD patients with DUOX2 gene variant carriage, and insights into a preclinical pipeline to test candidate molecules regulated by the microbiota including primary and secondary bile acids.

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