Deficits in gastrointestinal (GI) epithelial barrier function play important roles in the pathogenesis of Inflammatory Bowel Disease (IBD). The CD1H gene encoding E-cadherin, a key component of the epithelial junctional complex, is associated with Ulcerative Colitis (UC), and perhaps Crohn's disease (CD). E-cadherin is the principle adhesive component of the adherens junction, and it regulates paracellular permeability by facilitating the formation of tight junctions and organizing the entire epithelial junction complex. We have identified monoclonal antibodies (mAbs) that bind to E-cadherin and activate adhesion in a variety of epithelial cells. In this study, we aim to test the hypothesis that strengthening E-cadherin adhesion with activating mAbs will enhance barrier function and decrease progression of IBD while maintaining mucosal health and homeostasis. Mouse mAbs to E-cadherin have been tested in vivo using the IL10-knock out mouse and adoptive T cell transfer model of colitis with similar histological evaluation. Transfer of CD4+CD45RB high T cells from donor to immunocompromised mice produced typical histologic lesions for the adoptive transfer model including inflammation of the mucosa/submucosa, crypt damage, erosions, edema, and epithelial hyperplasia. E-Cadherin activating mAb (566-4) treatment reduced total colitis score, mucosal hyperplasia, inflammation, gland loss scores, and neutrophil infiltration in CD45RB+ high T cell recipient mice compared to control-e cad mAb (r19.1–10) treatment. In vivo, IL10 KO mice developed spontaneous severe inflammation and severity scores were lower in the r19.1–10 treatment group in comparison to the r19.1–10 treatment group for all the histological hallmarks of colitis. Further studies are in progress to investigate the therapeutic potential of E-Cadherin mAbs in the rescue of inflammation in pre-clinical mouse models of colitis.

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EPITHELIAL-DERIVED Selenoprotein P PROTECTS FROM COLITIS-ASSOCIATED CARCINOGENESIS

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Patients with inflammatory bowel disease demonstrate selenium nutritional deficiencies and are at increased risk for colon cancer. Previously, we determined that global loss of epithelial-derived selenoprotein P (SELENOP), exacerbates experimental colitis-associated cancer (CAC). Using a variety of tissue-specific knockout mouse lines, we further determined that epithelial-derived SELENOP served as the primary mediator of disease severity, and Villin-Cre;Selenopf/f (Selenop ΔE) mice have increased tumor incidence, number-size, and dysplasia as compared to WT cohorts. We next aimed to establish the mechanism by which epithelial-derived SELENOP modifies inflammatory tumorigenesis. We first investigated SELENOP’s effect on tumor promotion, and although SELENOP ΔE mice were larger, tumor cell proliferation was unchanged. Next, we evaluated the effect of selenoprotein P on mitochondrial respiration in an inflammatory environment. In this study, we demonstrate that global loss of epithelial-derived SELENOP modifies inflammatory tumorigenesis. We next hypothesized that loss of epithelial-derived SELENOP modifies tumor initiation, and in support of this, an analysis of endoscopic videos identified increased tumor numbers at early stages of experimental carcinogenesis. Tumors from Selenop ΔE mice also showed increased staining for markers of apoptosis and DNA damage, suggesting earlier tumor initiation may be due to increased oxidative stress. Further, myeloid-specific Selenop knockout, which did not alter inflammatory tumorigenesis, upregulated other antioxidant selenoproteins which may compensate for SELENOP loss, but this increase was not observed in the Selenop ΔE model. Interestingly, querying of the Predicting Response to Standardized Pediatric Colitis Therapy (PROTECT) cohort indicated that SELENOP is downregulated in UC tissues, with greater downregulation in severe disease, but levels of other key antioxidant selenoproteins were again unchanged. Finally, to more clearly investigate SELENOP loss in epithelial cells, SELENOP was knocked down in human adult ulcerative colitis organoids. Here, knockdown induced a pronounced growth defect, increased apoptosis, and increased oxidative stress as measured by Cellflour staining intensity. Together, these results suggest a model in which epithelial SELENOP protects from CAC by buffering oxidative stress in colonic epithelial cells in the setting of an inflammatory microenvironment.

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IN SILICO IDENTIFICATION OF PUTATIVE CLAUDIN CHANNEL BLOCKERS

Emma Wu, Priyanka Samanta, Ye Li, Le Shen, Fatemeh Khalili, Christopher Weber

Compromised epithelial barrier function is known to be associated with inflammatory bowel disease (IBD) and may contribute to disease development. One mechanism of barrier dysfunction is increased expression of paracellular tight junction ion and water channels formed by claudins. Claudin-2 and -15 are two such channels. We hypothesize that blocking these channels could be a viable therapeutic approach to treat diarrhea in IBD. In an effort to develop blockers of these channels, we turn to our previously developed and validated in silico models of claudin-15 (Samanta et al. 2018). We reasoned that molecules that can bind with the interior of claudin pores can limit paracellular water and ion flux. Thus, we used docking algorithms to search for putative drugs that bind in the claudin-15 pore. AutoDock Vina (Scripps Research Institute) was initially used to assess rigid docking using small molecule ligand databases. The ligands were analyzed based on binding affinity to the pore and visualized using VMD (University of Illinois at Urbana-Champaign) for their potential blocking of the channel. Overall, a total of eight candidate ligands from the databases were identified: three from the UCentre database of 10000 ligands, one chemically similar structure identified in another online database (Chemspider), and four which are modifications on the chemical structure generated using ChemDraw. The analysis revealed that the eight ligands were docked in two predominant positions. In the first position, the ligands with more rings docked in an almost linear fashion and interacted with both DSS and D64 pore residues. In the second position of binding, the ligands were more flexible and could hence fold to interact only with DSS residues, thus binding predominately in the center of the pore. To further evaluate these ligands, we will now turn to 1) flexible claudin-15 docking studies, 2) molecular dynamic simulations, and 3) in vitro measurements using monolayers induced to express claudin -15 and -15-mutated claudins. We expect the optimization of these ligands will provide a platform towards designing new drugs to block claudin-2 and -15.

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MECHANISMS OF ACTION FOR THE IBD-RISK GENE C1ORF106/INAVA

Phi Luong, Wayne Lencer, Denis Chang, Qian Li

C1ORF106, also named INAVA (Innate Immune Activator), was identified as a risk gene for the chronic inflammatory bowel diseases (IBD) by genome-wide association studies. INAVA encodes a protein that is a component of the ER chaperone/adenosine triphosphatase (ATPase) complex. Using small molecule ligand databases. The ligands were analyzed based on binding affinity to the pore and visualized using VMD (University of Illinois at Urbana-Champaign) for their potential blocking of the channel. Overall, a total of eight candidate ligands from the databases were identified: three from the UCentre database of 10000 ligands, one chemically similar structure identified in another online database (Chemspider), and four which are modifications on the chemical structure generated using ChemDraw. The analysis revealed that the eight ligands were docked in two predominant positions. In the first position, the ligands with more rings docked in an almost linear fashion and interacted with both DSS and D64 pore residues. In the second position of binding, the ligands were more flexible and could hence fold to interact only with DSS residues, thus binding predominately in the center of the pore. To further evaluate these ligands, we will now turn to 1) flexible claudin-15 docking studies, 2) molecular dynamic simulations, and 3) in vitro measurements using monolayers induced to express claudin -15 and -15-mutated claudins. We expect the optimization of these ligands will provide a platform towards designing new drugs to block claudin-2 and -15.

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OXPHOS INDUCTION IN HEALING: A CRITICAL MISSING STEP IN INFLAMMATORY BOWEL DISEASE?

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Background: In Inflammatory Bowel Disease (IBD), mucosal healing represents a key outcome in clinical remission. Mitochondrial dysfunction is one of the major features of IBD, which is hallmark by increased oxidative stress and impaired ATP production. However, partial wound healing continues even with repressed mitochondrial respiration in an inflammatory environment. In this study, we demonstrate that intestinal epithelial cells (IEC) responding to colitis produce a gene signature with downregulated mitochondrial expression associated with oxidative phosphorylation (OxPHOS) and upregulate epithelial-to-mesenchymal transition (EMT). Our data were consistent with the notion that EMT induced in IEC is fueled by glycolysis (repressed OxPHOS). We also wish to determine if this OxPHOS repression is present in normal healing.

Methods: We are actively enrolling patients with Ulcerative Colitis (UC) and normal patients into a prospective study evaluating biopsy site healing. Biopsy samples from UC patients were collected in AllProtect® or PBS. Biopsy samples collected in PBS were processed for IEC isolation using enzymatic digestion followed by flow sorting of EpCAM+ cells. To investigate the healing process in colitis and normal patients, we utilized a sigmoid colon in a rat fed a high fat-low fiber diet. IEC were isolated and cultured in AllProtect® or PBS. Results: Genes corresponding to subunits of NADH dehydrogenase (Ubiquinone) of complex I (ND1, ND2, N3D, ND4, NDS and N6D) were consistently downregulated