consistently altered in IBD patients before the onset of disease. However, the involvement of DUOX2 in IBD is not well understood. We aimed to define how inflammation and the microbiota regulate DUOX2 activity.

Methods: C57Bl/6J males raised in specific-pathogen free (SPF) and germ-free (GF) conditions underwent a model of dextran sulfate sodium (DSS)-induced colitis for 6 days. We obtained colon specimens for histopathology and isolation of colon epithelial cells (CEC). We stimulated colonoids from wild-type (WT), toll-like receptor 4-KO (*Tlr4*-KO), and *Duox2*-KO mice with IFNγ, heat-killed adherent invasive Escherichia coli (AIEC) strain LF82, and heat-killed *F. prausnitzii* strain A2-165 and determined expression of *Duox2*, *Duoxa2*, and production of H_2O_2. We also treated colonoids with butyrate, a microbial metabolite with anti-inflammatory properties. Extracellular H_2O_2 production was analyzed by means of the Amplex Red assay, whereas gene expression was determined by qPCR.

Results: SPF mice undergoing DSS-induced colitis developed overt inflammation that was accompanied by upregulation of *Duox2* and *Duoxa2*, as well as increased production of H_2O_2 in freshly isolated CECs. DSS-treated GF mice developed a mild inflammation that also caused increased H_2O_2 production and *Duoxa2* upregulation. WT colonoid stimulation with IFNγ and the Crohn's disease-associated pathobiont AIEC induced *Duox2* and *Duoxa2* expression, whereas heat-killed *F. prausnitzii* did not. Similarly, both IFNγ and AIEC promoted epithelial production of H_2O_2 in WT colonoids but not *Duox2*-KO colonoids, indicating that epithelial release of H_2O_2 in response to these stimuli is mediated by DUOX2. Response to AIEC additionally required functional TLR4. Although heat-killed *F. prausnitzii* did not alter H_2O_2 production, its metabolite butyrate caused a significant blockade in the release of H_2O_2 in response to both IFNγ and AIEC.

Conclusions: Our results show that both inflammation and pathobionts induce the expression and activity of DUOX2, which begets more inflammation. We posit that specific depletion of pathobionts or restitution with butyrate-producing bacteria such as *F. prausnitzii* may be beneficial in IBD.

LOSS OF GLUTATHIONE PEROXIDASE 1 ATTENUATES COLITIS AND IS CRITICAL IN ACTIVATING EPITHELIAL REGENERATIVE RESPONSES

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Many selenium-containing "selenoproteins" function as antioxidants, and work by our lab and others has demonstrated that selenoproteins often protect against intestinal inflammatory diseases, including colitis. Glutathione peroxidase 1 (GPx1) is a ubiquitous, mitochondrial and cytosolic selenoprotein which catalyzes the reduction of hydrogen peroxide by glutathione. Previously, we determined that despite its antioxidant role, loss of GPx1 greatly reduced disease severity in the dextran sodium sulfate (DSS) colitis model. Furthermore, GPx1 loss increased baseline intestinal cell proliferation, enhanced enteroid plating efficiency, and induced expression of stem cell-associated genes, such as *Lgr5*.

Next, we aimed to determine the mechanism by which GPx1 modifies response to DSS. We observed that GPx1 is increased in colonic tissues from DSS-treated mice as compared to nontreated controls, suggesting that GPx1 may functionally contribute to intestinal injury responses. While GPx1 is expressed in both intestinal epithelial and immune cells, *in situ* hybridization to visualize *Gpx1* identified epithelial cells as the most highly expressing cell type, with the greatest *Gpx1* upregulation observed in wound-adjacent and regenerative crypts. Next, we investigated whether GPx1 loss affects stem cell function after injury. Here, we determined that both proliferation ($p<0.01$) and *Lgr5* expression ($p<0.05$) were increased in the crypts of *Gpx1-/-* DSS-treated mice in comparison to WT controls. Similarly, organoids established from ulcerative colitis tissue displayed increased growth rates ($p<0.01$), expression of stem cell and Wnt target genes such as *AXIN2* ($p<0.0001$) and *LGR5* ($p<0.01$), and proliferation ($p<0.05$) following *GPX1* knockdown. Together, these results indicate that GPx1 has an epithelial-cell autonomous role, and that its loss activates stem cell and proliferative responses which may both protect from intestinal injury and promote healing.

Interestingly, recent research has highlighted the role of cellular metabolism in maintaining intestinal stem cell function, and GPx1 has previously been implicated in these processes. RNA-sequencing from DSS-treated mice and gene set enrichment analysis identified a positive association with oxidative phosphorylation-associated genes in *Gpx1-/-* mice (NES: 1.78; FDR q-val: 0.01), suggesting altered metabolism which may favor stem cell function. Further analysis of cellular metabolism using *GPX1* knockdown colorectal cancer cells observed higher basal respiration ($p<0.0001$) and ATP generation ($p<0.0001$). Together, these results suggest that unlike other intestinal selenoproteins studied to date, loss of GPx1 augments stem cell injury responses to protect against intestinal inflammation, likely via augmenting epithelial regenerative responses.

METHYLTRANSFERASE SMYD5 EXAGGERATES INFLAMMATORY BOWEL DISEASE BY REGULATING PPAR-γ COACTIVATOR 1-α STABILITY

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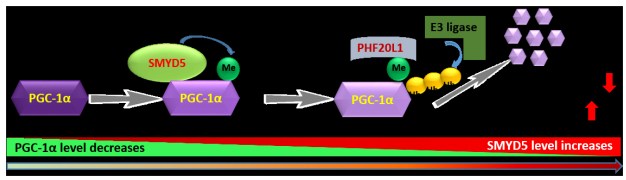
Background and Aims: The expression and role of methyltransferase SET and MYND domain-containing protein 5 (SMYD5) in inflammatory bowel diseases (IBD) is completely unknown. Here, we investigated the role and the underlying mechanism of epithelial SMYD5 in IBD pathogenesis and progression.

Methods: The expression and subcellular localization of SMYD5 and peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) were examined by Western blot analysis, immunofluorescence staining, and immunohistochemistry in intestinal epithelial cells (IECs) and in colon tissues from human IBD patients and mice with experimental colitis. Mice with Smyd5 conditional knockout in IECs and littermate controls were subjected to DSS-induced experimental colitis and the disease severity and inflammation were assessed. SMYD5-regulated mitochondrial biogenesis was examined by RT-qPCR and transmission electron microscopy and mitochondrial oxygen consumption rate was measured in a Seahorse Analyzer system. The interaction between SMYD5 and PGC-1α was determined by co-immunoprecipitation assay. PGC-1α degradation and turnover (half-life) were analyzed by cycloheximide chase assay. SMYD5-mediated PGC-1α methylation was measured via *in vitro* methylation followed by mass spectrometry to identify the specific lysine residues that were methylated.

Results: Up-regulated SMYD5 and down-regulated PGC-1α were observed in IECs from IBD patients and mice with experimental colitis. However, Smyd5 depletion in IECs protected mice from DSS-induced colitis. SMYD5 was critically involved in regulating mitochondrial biology such as mitochondrial biogenesis, respiration, and apoptosis. Mechanistically, SMYD5 regulated mitochondrial functions in a PGC-1α dependent manner. Further, SMYD5 mediated lysine methylation of PGC-1α and facilitated its ubiquitination and proteasomal degradation.

Conclusion: SMYD5 attenuates mitochondrial functions in IECs and promotes IBD progression by enhancing the proteasome-mediated degradation of PGC-1α protein in a methylation-dependent manner. Strategies to decrease SMYD5 expression and/or increase PGC-1α expression in IECs might be a promising therapeutic approach to treat patients with IBD.



PROTON PUMP INHIBITORS (PPI) INDUCES COLONIC TIGHT JUNCTION BARRIER (TJ) DYSFUNCTION VIA AN UPREGULATION OF TJ PORE FORMING CALUDIN-2 PROTEIN

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Background: Proton pump inhibitors (PPIs) are highly effective antagonists of gastric acid secretion and are widely used to treat a number of gastroesophageal disorders, including peptic ulcer disease, GERD, and Barrett's esophagus. PPI-induced elevation in intra-gastric pH and subsequent alterations of gastrointestinal physiology are thought to cause undesired effects on the entire GI tract. Defective intestinal Tight Junction (TJ) barrier is an important pathogenic factor for intestinal inflammation. Claudin-2 is a pore forming TJ protein whose overexpression causes selective increase in TJ permeability to small molecules. Claudin-2 expression is known to be up-regulated in intestinal inflammation and inflammation-associated colon carcinogenesis. The effect of PPI on Intestinal TJ barrier is not known. Aim: The aim of the present study was to study the effect of PPI on Intestinal TJ permeability.

Methods: A cell culture model of filter grown human intestinal epithelial Caco-2 monolayers, was used to study intestinal epithelial TJ barrier function. The mouse colonic permeability was measured by Ussing chambers studies. Western blot (WB) and Immunofluorescence (IF) was used to study the protein expression of claudin-2 in Caco-2 cells and in mouse colon. Cell surface biotinylation was used to study intracellular trafficking of TJ proteins.

Results: PPIs, caused a concentration- and time-dependent decrease in transepithelial resistance (TER), and increase in urea flux in filter-grown Caco-2 cells. Further studies on intestinal TJ revealed that PPI caused a significant increase in pore forming TJ protein claudin-2 protein level (western blot), but not mRNA (RT-PCR) in Caco-2 monolayers. Claudin-2 was co-localized to its known intercellular trafficking vesicles, clathrin pits. PPI treatment caused an increase in expression and junctional localization of claudin-2 and a decrease in its cytoplasmic co-localization with clathrin. Similarly, pulse-chase assay showed that the claudin-2 half-life was increased by PPI. In cell surface biotinylation assay, the rate of claudin-2 endocytosis was significantly reduced by PPI. In ex-vivo, Ussing chamber studies, PPI administration for 45 days resulted in a significant decrease in murine colonic TER and increase in urea flux. Immunofluorescence and Western blot analysis showed that PPI also caused an increase in TJ protein claudin-2 expression in mice colonic mucosa.

Conclusion: Our results suggest that the PPIs induces colonic TJ permeability via increasing pore forming claudin-2 levels. PPIs affect intracellular trafficking to reduce claudin-2 degradation and increased its junctional localization.