A035 Butyrate Regulates the Actin-Binding Protein Synaptopodin to Promote Wound Healing and Intestinal Epithelial Barrier Function

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Introduction: Intestinal epithelial cells (IECs) form a dynamic barrier that maintains homeostasis by partitioning the host immune system from luminal contents, including the microbiota. Disruption of epithelial barrier in diseases such as inflammatory bowel diseases (IBD) can increase bacterial translocation and result in inappropriate immune activation. During disease flares, rapid wound healing for barrier restoration is central to the containment of inflammation and the initiation of resolution. Mucosal barrier disruption is often associated with dysbiosis, particularly decreases in species producing short chain fatty acids (SCFAs). While SCFAs are an established energy source for IECs, we hypothesized that microbial-derived SCFAs could promote IEC barrier function through specific gene regulation. **Methods and Results:** The SCFA butyrate was shown to selectively augment barrier formation and enhance wound healing in T84 model IEC monolayers. An unbiased single cell RNA sequencing analysis (scRNAseq) was performed to define potential mechanisms of butyrate regulation and identified a number of epithelial barrier function coordinating gene targets. Of particular interest was the prominent butyrate-induced expression of synaptopodin (SYNPO), an actin-associated protein involved in cell shape and motility previously shown to be expressed in neuronal dendrites and kidney podocytes. Validation of the scRNAseq revealed that butyrate induces IEC SYNPO mRNA and protein expression by nearly 10-fold in IECs, and that lentiviral knockdown of SYNPO results in diminished barrier formation and wound healing not rescued with butyrate treatment. Immunofluorescence studies revealed that SYNPO distinctly localizes to the IEC tight junction (e.g. co-localization with ZO-1), the linkage between cells responsible for regulating paracellular flux. *In vivo* studies showed that mice subjected to dextran sulfate sodium (DSS)-induced colitis have decreased SYNPO expression. *Synpo*-deficient mice demonstrated exacerbated disease susceptibility and increased intestinal permeability in the DSS colitis model. **Conclusion:** These findings establish a critical role for the microbiota and their products, specifically butyrate, in the regulated expression of SYNPO for intestinal homeostasis and reveal a direct mechanistic link between microbiota-derived butyrate and barrier restoration. Understanding butyrate regulation of barrier function in the intestinal mucosa may offer insight into IBD therapeutics to promote wound healing.