MAJOR GENE REGULATORS AFFECTED IN COLON AND BLOOD OF DEXTRAN SODIUM SULFATE ACUTE COLITIS MURINE MODEL

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Background: Dextran sulphate sodium (DSS) ulcerative colitis (UC) murine models have long been used for in vivo studies. DSS is a negatively charged polysaccharide with colitogenic properties. Although the mechanisms by which DSS induces intestinal inflammation are not fully understood, there are several good reasons why the DSS chemical colitis model for investigating the immunopathogenesis of UC is widely used. These include strong phenotypic clinical manifestations which emulate numerous clinical and histopathological features of human UC, ease of use, low mortality rate and high reproducibility. Here, by using high-throughput RNA sequencing analysis we set to investigate major predicted gene regulators (GRs) affected by differentially expressed genes in the DSS treated UC model in order to obtain regulatory insights into the pathogenic mechanisms of UC development.

Methods: A DSS-induced mouse model of UC was established. Total RNA from colon tissue and blood of 3 healthy and 3 DSS-treated mice was extracted and sequenced by Illumina HiSeq 4000. Gene expression levels were obtained by mapping and quantification to the annotated mouse genome. Subsequently, differential gene expression analysis between DSS-treated and control mice both in colon and blood was performed. Ingenuity pathway analysis software (IPA®, Qagen) was used to predict/identify major GRs affected by significantly differentially expressed genes (SDEGs, FC > 2; p ≤0.05) in both colon and blood.

Results: Our analysis revealed how many and which major GRs are affected in DSS-treated mice and also the direction of change as compared to healthy (untreated) mice. In colon, 595 activated and 198 inhibited major GRs (p-value of overlap ≤0.05) in relation to ~3180 SDEGs were identified, while in blood, we identified 205 activated and 62 inhibited GRs (in relation to ~650 SDEGs). Colon and blood share 181 activated and 41 inhibited GRs. Identified GRs include transcription regulators, cytokines, transmembrane receptors and enzymes that mainly contribute to the development of inflammatory/immune responses. In colon and blood, the top 10 activated and inhibited regulators with the highest positive and negative activation z-score with target molecules as well as expression in the datasets are indicated in Figure 1a and 1b, respectively.

Conclusion: In this study, we analyzed linkage of GRs to SDEGs through coordinated expression and identified potential major regulators that have significant effect on UC pathogenesis-related gene expression. These GRs seem to be the key regulators of transcriptomic changes induced by inflammation. These findings expand our molecular understanding of putative new targets that may be important in the pathophysiology of UC and provide biological insights into the observed expression changes between the UC and healthy controls.

Figure 1. Enrichment significance of GRs that can regulate UC pathogenesis in DSS model in (a) colon and (b) blood. An overlap right-tailed fisher’s exact test p-value is computed based on significant overlap between genes in the dataset and known targets regulated by the regulators. Based on ingenuity knowledge base annotation, the activation z-score of GR is computed by the regulation direction associated with the relationship from the regulator to the gene.

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SCGN DEFICIENCY RESULTS IN COLITIS SUSCEPTIBILITY

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Inflammatory bowel disease (IBD) affects 1.5–3.0 million people in the United States. IBD is genetically determined and many common risk alleles have been identified. Yet, a large proportion of genetic predisposition remains unexplained. In this study we report the identification of an ultrarare missense variant (NM_006998.3:c.230G>A;p.Arg77His) in the SCGN gene causing Mendelian early-onset ulcerative colitis. SCGN encodes a calcium sensor that is exclusively expressed in the gut and neurons. SCGN interacts with the SNARE complex, which is required for vesicle fusion with the plasma membrane. We show that the SCGN mutation identified impacted the localization of the SNARE complex partner, SNAP25, leading to impaired hormone release. Finally, we show that mouse models of Scgn deficiency recapitulate impaired hormone release and susceptibility to DSS-induced colitis. Altogether, these studies demonstrate that functional deficiency in SCGN can result in intestinal inflammation and implicates the neuroendocrine cellular compartment in IBD.

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A FUNCTIONAL TL1A/DR3 SYSTEM IS CRUCIAL FOR OPTIMAL GENERATION OF PATHOGENIC TH9 CELLS IN MURINE CROHN’S DISEASE-LIKE ILEITIS

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Background: Despite numerous therapeutic advancements, inflammatory bowel disease (IBD) remains a major health burden due to the inefficiency of conventional