SZN-1326 Promotes Colonic Mucosal Healing in an Acute Injury Model of IBD by Expanding the Progenitor Pool and Accelerating Differentiation (3525628)

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Introduction

Wnt signaling plays critical roles in regulating intestinal stem cell maintenance and differentiation. Loss or inhibition of Wnt signaling leads to the loss of intestinal crypts due to a reduction in stem and progenitor cell proliferation and self-renewal, and, conversely, constitutive activation of the pathway leads to hyperproliferation (Mah 2016). The inflammatory bowel disease, ulcerative colitis (UC), is characterized by epithelial lesions and immune cell infiltration, and there is a need for therapies that promote mucosal healing in addition to limiting inflammation (Chang 2020). Previously, we have shown that our Wnt signaling agonist mimetic molecules, termed Surrozen Wnt signaling Agonist Proteins (SWAPs), were capable of robustly promoting stem and progenitor cell expansion in intestinal organoids (Chen 2020). SZN-1326 is a FZD5,8 and LRP6-specific, full-length, effectorless, bi-specific IgG1 molecule that is well-suited for impacting the intestinal epithelium where FZD5 is highly expressed. We have tested SZN-1326 in the acute DSS mouse colitis model, and SZN-1326 ameliorates colitis. To gain a more comprehensive understanding of the mechanism of action of SZN-1326 in promoting colonic epithelial repair and mucosal healing we applied a range of approaches including scRNA-seq to interrogate how transient Wnt activation with SZN-1326 affects the colon and leads to healing in the acute DSS model.

Materials and Methods

We employed the acute dextran sodium sulfate (DSS) epithelial injury model, where 4% DSS is provided in the drinking water for seven days followed by 1% DSS until termination at day 10. Animals were dosed on day 4 with the SWAP, SZN-1326, or an Anti-GFP antibody at 10 mpk, and we subsequently applied scRNA-seq (10X Genomics 3', v3 reagents) to all tissue layers of the adult mouse colon in this model at day 5 and day 6 (24- and 48-hours after dosing). We also collected tissue for histology and RT-qPCR. In a second approach, animals were dosed with SZN-1326 or Anti-GFP at day 4 and day 7 and terminated at day 10.

Figure 1.

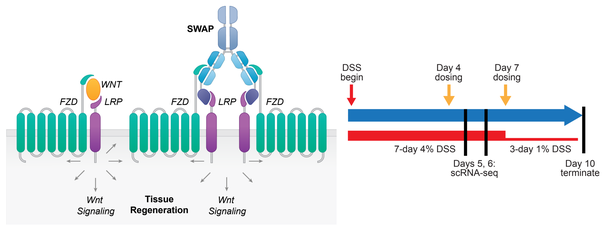
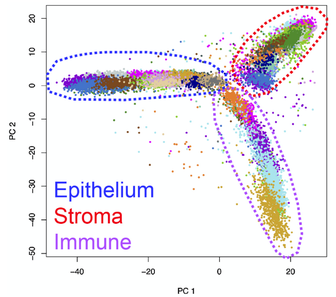


Figure 2.

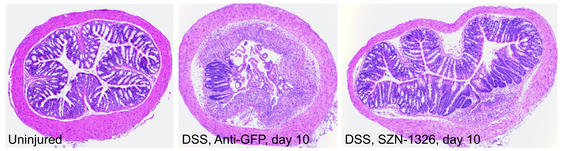


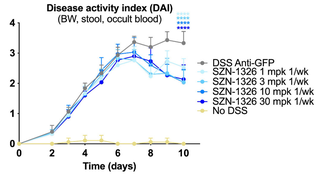
After filtering, there are 4835 immune cells, 7509 stromal cells, and 10373 epithelial cells. The different tissue layers/lineages radiate away from each other in a plot of the first two principal components (PC) (left).

Results

SZN-1326 leads to mucosal healing in the acute DSS colitis model

Figure 3.

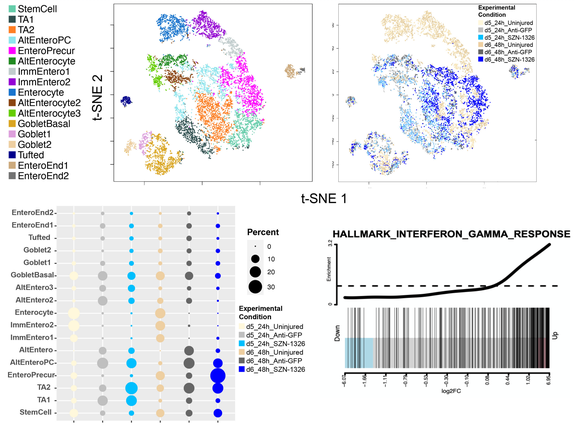




Cross sections of the transverse colon in the indicated conditions highlight that SZN-1326 improved the histology (top row), and SZN-1326 significantly improves the disease activity index.

DSS damage results in injury-specific cell types, including altered enterocytes and progenitors

Figure 4.



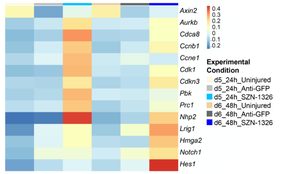
In the t-SNE plots of the epithelial cells from the scRNA-seq analysis, the cell type annotations are indicated in the left plot, and the experimental condition of the cells is indicated on the right. The bubble plot shows the percentage of cells from each experimental condition present in a given cell type. Normal enterocytes are only present in the uninjured condition. In the DSS damage model, the enterocytes are altered to injury-induced states (AltEnterocyte, AltEnteroPC), expressing inflammation associated genes and showing enrichment for pathways such as interferon gamma signaling (barcode plot of the AltEnterocytes).

The predominant impact of SZN-1326 is on the epithelium where it induces Wnt target and cell cycle gene expression and expands the progenitor pool

Figure 5.

Timepoint: Tissue	DSS vs Uninjured DEG	SZN-1326 vs Anti-GFP DEG
Day 5, 24h: Immune	2045	143
Day 6, 48h: Immune	2064	312
Day 5, 24h: Stroma	961	78
Day 6, 48h: Stroma	1017	181
Day 5, 24h: Epithelium	1880	1365
Day 6, 48h: Epithelium	1394	1475

DSS damage drastically impacts the number of differentially expressed genes (DEG) in all tissue layers/lineages, while the predominant effect of SZN-1326 is on the epithelium

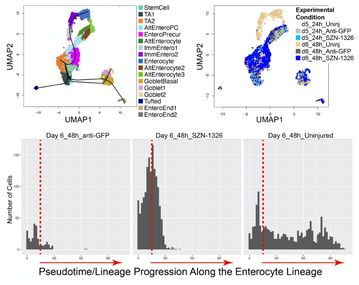


Pathway	FDR
REACTOME_POL_SWITCHING	0.000810222
HALLMARK_MYC_TARGETS_V1	0.000810222
REACTOME_EXTENSION_OF_TELOMERES	0.000810222
REACTOME_LAGGING_STRAND_SYNTHESIS	0.000810222
REACTOME_TELOMERE_MAINTENANCE	0.000810222
REACTOME_MITOTIC_G1_G1_S_PHASES	0.000810222
REACTOME_CELL_CYCLE_CHECKPOINTS	0.000810222
REACTOME_S_PHASE	0.000810222
REACTOME_G1_S_TRANSITION	0.000810222
HALLMARK_DNA_REPAIR	0.000923239
SA_G1_AND_S_PHASES	0.000944084
KEGG_RNA_POLYMERASE	0.001398853
HALLMARK_MYC_TARGETS_V2	0.001398853
KEGG_PYRIMIDINE_METABOLISM	0.001620146
REACTOME_G1_S_SPECIFIC_TRANSCRIPTION	0.001655543
REACTOME_CELL_CYCLE_MITOTIC	0.002203089
REACTOME_PERK_REGULATED_GENE_EXPRESSION	0.006805139
REACTOME_SIGNALING_BY_WNT	0.016631866
HALLMARK_MTORC1_SIGNALING	0.029879153
HALLMARK_UNFOLDED_PROTEIN_RESPONSE	0.029879153

At 24-hours after dosing, we detect an expansion of Wnt target and cell cycle gene expression (heatmap shows a few examples). This is an increase in both the level of expression and the number of cells that express these genes. Pathway analysis shows that SZN-1326 increases expression of genes associated with the cell cycle, cell growth, telomere maintenance, Wnt signaling, and the unfolded protein response (Table, above).

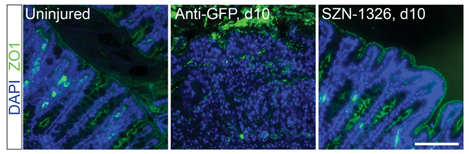
SZN-1326 leads to accelerated epithelial differentiation and barrier repair

Figure 7.



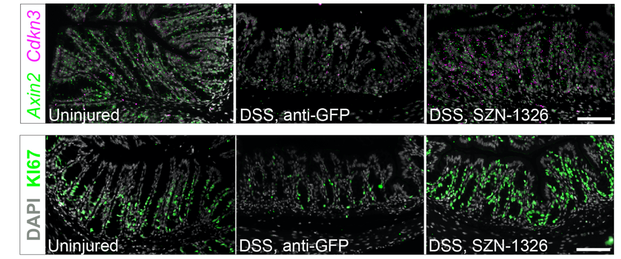
By assessing the real time-stamping of the cells (bubbleplot further to the left), we see that by 48-hours, there are more enterocyte precursors in the SZN-1326 treatment group relative to the Anti-GFP treated controls. To complement the real time-stamping, we applied the lineage trajectory inference tool, *slingshot*, to infer the epithelial lineage trajectories of cell types that were not injury-specific. Lineages are shown as the curves on the left uniform manifold approximation projection (UMAP) plot. *slingshot* predicts that the SZN-1326 cells are accelerated along the path to enterocyte differentiation by day 6 (48-hours after dosing), indicated by the increase in the number of cells further to the right in the histograms of lineage progression.

Figure 8.



In line with accelerated differentiation and barrier repair, expression of the tight junction marker, ZO1, is increased and more organized by day 10 in the SZN-1326 treatment relative to controls. Scale bar = 100 microns.

Figure 6.



By RNA in situ hybridization, we confirmed an expansion of the Wnt target genes, *Axin2* and *Cdkn3*, at day 5 by SZN-1326 treatment. Immunohistochemistry for the proliferative gene, Ki-67, shows that SZN-1326 expands the number of progenitors in the DSS damage model. Scale bars = 100 microns

Conclusions

- **SZN-1326 robustly induced intestinal epithelial healing**
- **DSS damage resulted in cells with injury-specific states, including progenitors**
- **SZN-1326 predominately impacted the epithelium where it induced Wnt target and cell cycle gene expression in the stem and progenitor cell populations**
- **SZN-1326 transiently expands the proliferative progenitor pool, but then the cells differentiate more quickly relative to the control-treated samples**
- **SZN-1326 ultimately leads to enhanced epithelial regeneration and barrier repair**

References and Acknowledgements

Mah et al. Wnt pathway regulation in intestinal stem cells. *J Physiol,* 594(17):4837-47 (2016)

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