INVESTIGATING DYSMOTILITY AND THE EXPANSION OF GLIAL CELLS AND INTERSTITIAL CELLS OF CAJAL IN CROHN'S DISEASE STRICTURES USING A NOVEL SURGICAL MOUSE MODEL

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Introduction

Crohn's disease (CD) is a type of inflammatory bowel disease (IBD) that can affect the entire digestive tract. One of the most significant complications of CD is intestinal stricture formation, which may cause bowel obstruction and dysmotility[1]. Nerve hyperplasia has also been identified as a prominent feature of CD[2], but the effect of these changes in the enteric nervous system (ENS) is still unclear, especially in the context of strictures. To understand how strictures disrupt gut motility, we utilized a novel surgical mouse model of intestinal strictures to study changes in the ENS, providing insight on the role of the ENS in intestinal fibrosis.

Methods

Surgical mouse model

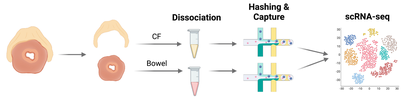
C57/B6 mice (n=3) with anti-mesenteric colotomies along with sham controls were harvested at POD 14. Frozen sections were stained by hematoxylin and eosin (H&E) and Masson's trichrome (TC).

Immunofluorescence (IF) staining

Frozen sections of intestinal strictures from pediatric CD patients, controls from ileostomy takedowns, mouse colotomy bowel, and mouse sham bowel were stained for markers of glial cells (GFAP) and interstitial cells of Cajal (cKIT).

Single-cell RNA-sequencing and CellChat

Cells from bowel and creeping fat were isolated separately from pediatric CD strictures (n=4) and sequenced.



Results

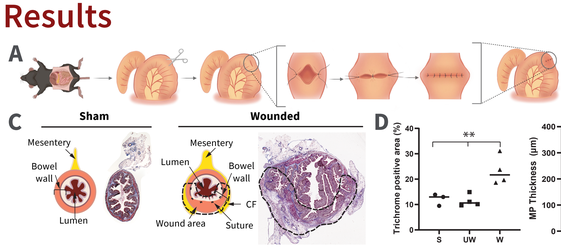


Figure 1: Surgical mouse model phenocopies human strictures and intestinal fibrosis. **A)** Schematic of mouse model. **B)** H&E staining showing increased muscularis propria (MP) thickness in wounded with the outlined area representing the stricture site. **C)** TC staining with schematics of sham and wounded with the outlined area emphasizing thickened muscle in wounded. **D)** Quantification of collagen deposition and MP thickness in sham (S), unwounded (UW), and wounded (W). **E)** Gross histological images showing the presence of creeping fat in wounded and outlined areas representing the unwounded/wounded site, respectively.

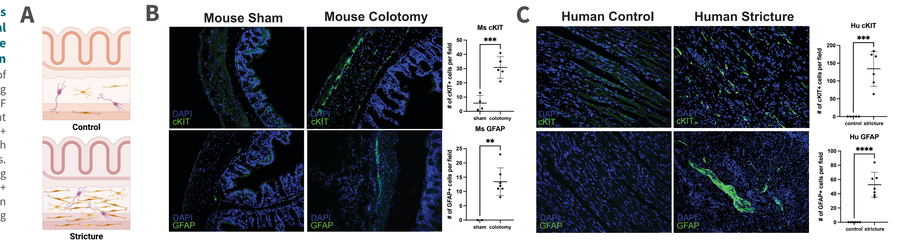


Figure 2: Interstitial cells of Cajal (ICCs) and glial cells expand in mouse colotomies and human strictures. **A)** Schematic of changes in ENS comparing control and wounded. **B)** IF staining showing significant increase in cKIT+ and GFAP+ cells in mouse colotomy with corresponding quantifications. **C)** IF staining showing significant increase in cKIT+ and GFAP+ cells in human stricture with corresponding quantifications.

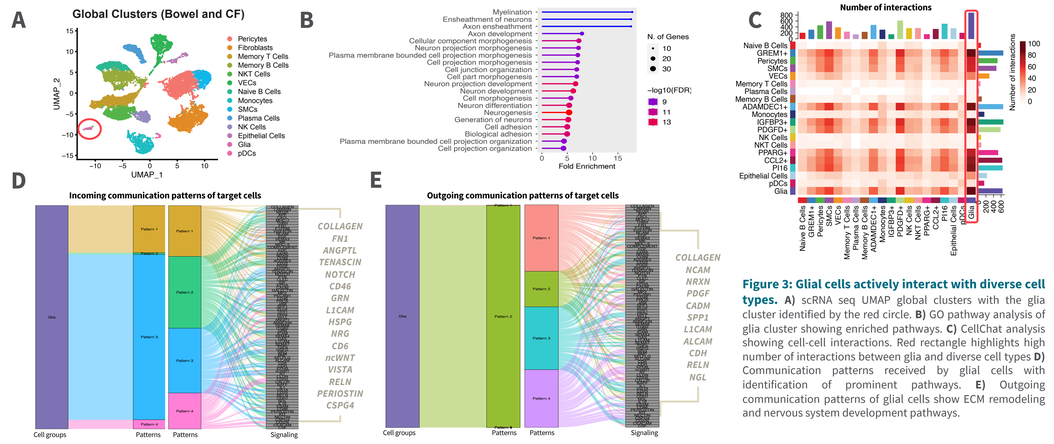


Figure 3: Glial cells actively interact with diverse cell types. **A)** scRNA seq UMAP global clusters with the glia cluster identified by the red circle. **B)** GO pathway analysis of glia cluster showing enriched pathways. **C)** CellChat analysis showing cell-cell interactions. Red rectangle highlights high number of interactions between glia and diverse cell types **D)** Communication patterns received by glial cells with identification of prominent pathways. **E)** Outgoing communication patterns of glial cells show ECM remodeling and nervous system development pathways.

Conclusions

Our surgical mouse model recapitulates the phenotype of human intestinal strictures, including the presence of thick smooth muscle, high levels of collagen deposition, and creeping fat. This novel mouse model of intestinal fibrosis may provide additional insights on the mechanisms of stricture formation.

There is an expansion of glial cells (GFAP+) and ICCs (cKIT+) in both mouse colotomy and human stricture. These findings suggest that a dysregulated ENS may promote dysmotility, especially since ICCs play a crucial role in gut motility and glial cells shape nerve development.

Our analysis shows that glial cells actively interact with diverse cell types, such as immune cells, smooth muscle cells, and fibroblasts. Analyzing both the incoming and outgoing communication patterns of glial cells, we identified several markers of interest that are involved in ECM remodeling[3], inflammation[4], and nervous system development[5].

Future Directions

Based on these preliminary findings, we aim to explore the connection between gut motility and glial cell/ICC expansion, focusing on the interactions between smooth muscle cells and ICCs. We will determine which pathways identified in our CellChat analysis drive nerve hyperplasia and attempt to inhibit them pharmacologically. Finally, we will explore the effects of nerve hyperplasia on gut motility.

References

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