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Development of a total colectomy and ileorectal anastomosis rat model to evaluate colonic metaplasia

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ABSTRACT

Background: Ulcerative colitis is an idiopathic inflammatory condition of the colon that may require surgical intervention including proctocolectomy and either ileal pouch-anal anastomosis or in the pediatric population, low ileorectal anastomosis (IRA). Often, subsequent physiologic alteration (or colonic metaplasia) occurs in the anastomosed small bowel that includes changes in mucin content, villous blunting, and increased expression of WNT5A, a marker of colonic crypt regeneration. We developed a rat low IRA model to assess and study the development of colonic metaplasia.

Materials and methods: We subjected male Sprague–Dawley rats ($n = 17$) to total colectomy and low IRA surgery and evaluated healing periodically by endoscopic evaluation. The ileum upstream of the anastomosis was assessed by hematoxylin and eosin staining, and the mucin content was measured by high iron diamine-Alcian blue staining. *Wnt5a* transcripts were quantified by reverse transcription and quantitative polymerase chain reaction at the 8-wk study end point.

Results: Although no gross endoscopic evidence of inflammation was seen throughout the course of the study, colonic metaplasia in the small bowel was detected in 7 out of 10 (70%) rats at the study end point. In rats with colonic metaplasia, enhanced expression of *Wnt5a* was evident at the study end point compared to levels in the terminal ileum at the time of surgery.

Conclusions: Within 4-8 wk, the majority of rats subjected to IRA developed colonic metaplasia defined by villous blunting, changes in mucin content, and increased expression of *Wnt5a*. This model provides a method to study small bowel colonic metaplasia.

Introduction

Ulcerative colitis is a type of inflammatory bowel disease that results from chronic inflammation of the colon, usually starting at the rectum and extending proximally.[1] The most common surgical procedure to treat ulcerative colitis in the adult is the formation of an ileal pouch-anal anastomosis (IPAA), which involves total proctocolectomy and formation of

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an ileal pouch from the terminal ileum.[2] However, in children, the creation of an ileal pouch is frequently unnecessary, so a low ileorectal anastomosis (IRA) just above the dentate line is performed.[3]

Consistent in both low IRA and IPAA procedures, however, is the occurrence of colonic metaplasia in the upstream ileum, possibly due to resulting inflammation.[4,5] Such colonic metaplasia can be identified by histopathological assessment of tissue sections and is defined by changes to the histologic architecture, including villous blunting and crypt hyperplasia.[6] Colonic metaplasia can be further defined by an alteration in the mucin content.[6] Mucins comprise the mucus barrier lining the epithelium of the intestine. They are highly glycosylated proteins that are terminally differentiated by sialic acid or sulfate, designated as sialomucin or sulfomucin, respectively.[7] The distribution of mucins differs spatially throughout the normal intestine, characterized by sialomucin in the small bowel and sulfomucin in the colon and rectum.[8] The presence of a mixed staining pattern or sulfomucin in the small bowel is abnormal and consistent with the development of colonic metaplasia.[9] Identification of sulfomucin in the ileum may be associated with nonspecific inflammatory processes rather than long-term adaptation.[10] Although changes in the mucin content have not been reported in IRA patients, patients with an ileocolonic resection for Crohn's disease demonstrated both villus irregularity and expression of sulfomucin in the upstream ileum.[11]

Acting through the noncanonical Wnt signaling pathway, Wnt family member 5a (WNT5A) stimulates a β-catenin-independent pathway to effect cell polarity and migration.[12] Following tissue injury, WNT5A has been shown to potentiate transforming growth factor beta levels to induce the formation of new colonic crypts and re-establish homeostasis.[13] Previously, a study reported that human ileal pouches demonstrated higher expression of *WNT5A*; however, the degree of inflammation or colonic metaplasia (measured by other techniques) was not reported.[14] The role of Wnt signaling has not been examined in IRA patients.

Better research is needed to understand the mechanism by which colonic metaplasia develops and how it may be related to the presence of inflammation and possibly pouchitis after IPAA surgery. Longitudinal analysis of low IRA or ileal pouches in the human is difficult and factors such as genetic susceptibility and variable environmental exposures confound such investigations. Therefore, the aim of this study was to develop a rat low IRA model to assess the development of colonic metaplasia in the ileum.

Materials and methods

Low ileorectal anastomosis animal model

This protocol was approved by the Pennsylvania State University College of Medicine Institutional Animal Care and Use Committee. Male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) ($n = 17$) were acclimated to housing facilities for at least 1 wk prior to surgery on 12-h light dark cycles. At the time of surgery, rats weighed between 250 g and 450 g. Animals were allowed food ad lib until surgery. Rats were anesthetized with a ketamine:xylazine mixture injected intraperitoneally at a dosage of 0.08 mL/10 g body weight. A small animal heating pad was used during surgery and in the postoperative recovery period (12 h) to maintain normothermia. While under anesthesia, the abdomen of the rat was shaved, cleansed with povidone iodine, and then rinsed with alcohol. Prior to the first incision, the rat was administered 10 mL of Lactated Ringer's solution (Baxter Healthcare, Deerfield, IL) subcutaneously in the scruff of the neck or hind quarters to counteract intraoperative fluid loss. A midline incision was made, and the terminal ileum transected proximal to the cecum. The ileocolic, right colic, middle colic, left colic, and sigmoid vessels were controlled using a handheld LigaSure Precise cautery (Medtronic, Minneapolis, MN). The rectum was transected approximately 0.5 cm from the anus. The entire colon, including the cecum, ascending, transverse, and descending segments, was removed. Stay sutures were placed at the transected rectum to prevent retraction. The end of the terminal ileum was anastomosed to the rectal stump using 8-10 interrupted 6-0 prolene sutures circumferentially. The midline incision was closed in two layers with running 5-0 nylon suture. After surgery, the rat was administered 1.0 mg/kg Buprenorphine SR (ZooPharm, Laramie, WY) subcutaneously to minimize postoperative pain and a second dose of 10 mL Lactated Ringer's subcutaneously.

Postoperative care

Rats were maintained in standard cages with a wire bottom to avoid contaminating the surgical site, prevent coprophagy, and consumption of the bedding. For the first night after surgery, rats were given water mixed 1:1 with Boost Breeze Nutritional Drink (Nestlé Health Science, Florham Park, NJ). Days 1-10 postoperation, the diet consisted only of a liquid purified low-residue diet (TD.160245, Envigo, Frederick, MD) prepared fresh every morning and water ad lib. At day 11 postoperative, rats were reintroduced to normal solid rat chow moistened with water, and on day 12 postoperative throughout the remainder of the study, rats were fed normal solid chow and water ad lib. Rats were killed at 8 wk postoperative by ketamine:xylazine overdose followed by exsanguination. The anastomotic site was dissected into three pieces and saved in RNAlater (ThermoFisher, Leesport, PA), fixed in formalin, and flash frozen in liquid nitrogen.

Endoscopy

Starting at day 14 postoperative and every 2 wk thereafter, endoscopy was performed on all rats. Rats were anesthetized with 30% (vol/vol) isoflurane using a nose cone. The rat was maintained on a small animal heating pad during endoscopy and throughout recovery to maintain normothermia. The anus was cleansed with povidone iodine and rinsed with alcohol. The Airway Mobilescope (MAF-GM, Olympus, Center Valley, PA) with an insertion tube outer diameter of 4.1 mm was covered in lubricating gel with 2% lidocaine and entered into the anus. At 4 wk, biopsies were obtained. The biopsy forceps (FB-34K-1, Olympus, Center Valley, PA) were covered in 2% lidocaine and biopsies taken above the IRA. Biopsies were fixed in formalin. A bowel preparation was not