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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 (UC) is an idiopathic inflammatory condition of the colon that may require surgical intervention including proctocolectomy and either ileal pouch-anal anastomosis (IPAA) 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 (H&E) staining and the mucin content was measured by high iron diamine-Alcian blue (HID-AB) staining. *Wnt5a* transcripts were quantified by RT-qPCR at the 8 week study endpoint.

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 endpoint. In rats with colonic metaplasia, enhanced expression of *Wnt5a* was evident at the study endpoint compared to levels in the terminal ileum at the time of surgery.

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Disclosure

None of the authors have any conflicts of interest or disclosures.

Conclusions—Within 4–8 weeks, 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.

Keywords

colonic metaplasia; ileorectal anastomosis; mucin; rat model

Introduction

Ulcerative colitis (UC) is a type of inflammatory bowel disease (IBD) that results from chronic inflammation of the colon, usually starting at the rectum and extending proximally.¹ The most common surgical procedure to treat UC in the adult is the formation of an ileal pouch-anal anastomosis (IPAA), which involves total proctocolectomy and formation of 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 non-specific 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 non-canonical 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 (TGF- β) 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 are 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 (IRA) animal model

This protocol was approved by the Pennsylvania State University College of Medicine Institutional Animal Care and Use Committee (IACUC). Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) ($n = 17$) were acclimated to housing facilities for at least one week prior to surgery on 12-hour 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 hours) 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 intra-operative 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 post-operative pain and a second dose of 10 mL Lactated Ringer's subcutaneously.

Post-operative 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 post-operation, 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 post-op, rats were re-introduced to normal solid rat chow moistened with water and on day 12 post-op throughout the remainder of the study, rats were fed normal solid chow and water *ad lib*. Rats were sacrificed at 8 weeks post-op 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 post-op and every 2 weeks thereafter, endoscopy was performed on all rats. Rats were anesthetized with 30% (v/v) 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 four weeks, 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 performed but introduction of sterile saline through the fluid feed channel port using a sterile syringe was used to distend the bowel and remove residual stool.

Immunohistochemistry

Formalin-fixed and paraffin-embedded sections of the low ileorectal anastomotic site and biopsies were cut and deparaffinized using a xylene and alcohol gradient (100% ethanol 3 min thrice, 95% 3 min twice, 70% ethanol 3 min once, and water 5 min). Slides were stained with hematoxylin and eosin (H&E) to evaluate the cellular architecture for villous blunting and crypt hyperplasia. Villus length was measured on H&E stained tissue sections at 4× magnification on the Olympus BH-2 microscope (Olympus, Center Valley, PA) using the AmScope MU500 (AmScope, Irvine, CA) and ToupView software. The first ten villi proximal to the suture line were measured as were ten villi from an area with normal ileal architecture, at least 2 cm from the anastomosis. To assess the mucin content, high iron diamine-Alcian blue (HID-AB) staining was performed. This stains sulfated mucins purple, usually only present in the colon and rectum, due to the iron content in the HID-AB stain. Sialylated mucins, commonly found in the small bowel, will stain blue by Alcian blue.¹⁵ Slides were incubated with high iron diamine for 16 hours followed by 1% Alcian blue in 3% acetic acid for 5 min.

Reverse transcription and quantitative PCR (RT-qPCR)

From tissue stored in RNeasy lysis buffer, RNA was first extracted using TRIzol reagent (ThermoFisher, Waltham, MA) and chloroform. As an additional purification step, the aqueous phase was transferred to an RNeasy MinElute spin column (Qiagen, Germantown, MD) and processed following the manufacturer's instructions. cDNA was synthesized using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA) from a total of 225 ng RNA. cDNA was diluted to 1:100 and gene expression was evaluated by quantitative real-time PCR using SensiFAST SYBR & fluorescein kit (BioLabs, Beverly, MA) as previously described.¹⁶ Primers used to detect *Wnt5a* were forward: ATCCCAGGTAGTTGAAGGGCAGAC and reverse: CCAGCAGAGTCCAGAGAGGGCTTT. *Actb* (beta actin) was used as an internal reference and the following primers were used, forward: AACACACGAGACGCTGAAGT and reverse: TCCAGTGAGTTCCGAAAGCC. Thermocycling conditions were 94°C for 3 min, 40 cycles of 94°C for 20 s and 68°C for 30 s, followed by a 4°C hold. Data is represented as relative expression using 2^{-C_t} .

Statistical analysis

Continuous variables were analyzed by two-tailed Student's *t*-test with significance determined by *P* < 0.05 using R software version 3.3.2 (The R Project for Statistical Computing, Vienna, Austria).

Results

Surgical outcomes

Rats were evaluated over a period of 8 weeks after the low IRA procedure (Fig. 1A). Construction of a low IRA and long-term evaluation of the upstream ileum resulted in few postoperative complications. Of the 17 rats subjected to the surgery, 4 (23.5%) were euthanized prior to the study endpoint due to surgical complications. Three (75%) of these four rats developed a volvulus of the bowel which resulted in obstruction and one (25%) stopped eating on day 23 post-op but laparotomy did not demonstrate a bowel obstruction. During the recovery period, we noted that heavier rats at the time of surgery tended to gain weight less rapidly (Fig. 1B). Rats weighing <350 g ($n = 13$, range 249–315 g) at the time of surgery had a greater total percent weight gain after 8 weeks ($68.6 \pm 29.9\%$) compared to those weighing >350 g ($n = 4$, range 380–460 g) at surgery ($18.3 \pm 11.6\%$) ($P < 0.001$). All rats gained less weight overall compared to a non-surgical control. For rats weighing < 350 g at surgery, weight loss was seen during the first 2–3 days after surgery. However, they gradually gained weight until day 10 when the liquid purified low-residue diet was switched to solid rat chow. Overall, a small weight loss was seen following the change from liquid to solid food but the animals steadily gained weight throughout the remainder of the study. While rats were on the liquid purified low-residue diet, the stool had the consistency of diarrhea; however, following the introduction of solid chow, the stool was excreted in compact pellets.

Cellular evidence of colonic metaplasia is present at 4 weeks

Starting 2 weeks post-op and biweekly thereafter, the anastomotic site and upstream ileum were evaluated by endoscopy to assess signs of mucosal inflammation using a MAF-GM scope (Fig. 2A, 2B). The rats tolerated the procedure well, with only one (5.9%) mortality due to anesthetic complications. The anastomotic site was visualized and the scope passed through the anastomosis into the ileum. None of the rats demonstrated gross endoscopic inflammation throughout the course of the study, as evident by an absence of erythema, ulceration, or mucous exudates (Fig. 2C). At 4 weeks, ileal biopsies were obtained upstream of the anastomosis and evaluated for villous blunting and a change in the mucin content. Villous blunting was apparent in biopsied sections as assessed by hematoxylin and eosin (H&E) staining (Fig. 2D). To evaluate the mucin content of the ileum, high iron diamine-Alcian blue (HID-AB) staining was performed. In the biopsied tissue sections, minimal to moderate levels of colonic sulfomucin were found (Fig. 2E). Therefore, although endoscopic evaluation did not detect gross inflammation throughout the study, cellular evidence of colonic metaplasia was evident in biopsied ileal sections at 4 weeks.

Villous blunting correlates with the presence of colonic metaplasia

At the study endpoint, 8 weeks following the IRA surgery, we collected intestinal tissue surrounding the anastomosis. To assess for colonic metaplasia, cellular architecture was evaluated by staining tissue sections with H&E. In some rats, severe metaplasia was seen, demonstrated by loss of normal intestinal architecture surrounding the anastomosis (Fig. 3A). However, moving proximally through the bowel from the anastomosis revealed normal ileal architecture further upstream, indicating that the colonic metaplasia was limited to 1–2

cm proximal to the anastomosis (Fig. 3B). The remaining 3 rats without significant differences in villus lengths demonstrated minimal villous blunting at the suture line that quickly recovered into normal ileal architecture (Fig. 3C). Villous blunting was quantitated by comparing villus length at the anastomosis and at normal healthy ileum within the same rat. In 8 out of 10 (80%) rats, significant villous blunting was found at the anastomosis at study endpoint relative to healthy ileum (Fig. 3D, Table 1).

Colonic mucin localizes to ileal tissue above the anastomosis

To further evaluate the presence of colonic metaplasia, we performed HID-AB staining to assess the mucin content of the epithelium. Correlating with the presence of villous blunting at the study endpoint, changes in the mucin content was present in 7 out of 10 (70%) rats (Table 1), as indicated by the presence of mixed sialomucin and sulfomucin found within goblet cells of the ileum at least 1 cm upstream of the anastomotic site (Fig. 4A, Supplemental Fig. 1). In those rats without mucin alterations, a clear demarcation of the ileum and rectum was seen (Fig. 4B).

Enhanced expression of *Wnt5a* mRNA indicates colonic crypt regeneration in the ileum

To assess for colon-like crypt regeneration in the ileum above the anastomosis, RT-qPCR was performed to measure the levels of *Wnt5a* gene expression. Rats with visual evidence of colonic metaplasia by both H&E and HID-AB demonstrated enhanced levels of *Wnt5a* gene expression within the ileum upstream of the anastomosis at the 8 week study endpoint relative to terminal ileum sections collected at the time of surgery (Fig. 5, Table 1). Conversely, rats without evidence of both measures of colonic metaplasia demonstrated minimal changes in *Wnt5a* expression.

Discussion

The development of an animal model to study colonic metaplasia will facilitate our understanding of inflammatory processes that occur as a result of IRA and IPAA surgeries. In the current literature, two groups have successfully performed IRA and ileal pouch-rectal anastomosis (IPRA) surgery on rats.^{17–19} In both Sprague-Dawley and Lewis rats with an ileal pouch^{17, 18} and Lewis rats with a straight IRA,¹⁷ increased ileal inflammation was seen at 4 and 8 weeks compared to non-operated ileum. Lichtman *et al.* found shortened villi in the ileal pouch of both Lewis and Sprague-Dawley rats after 4 weeks compared to non-operated controls; however, no mucin changes were present after 8 weeks.¹⁷ Stucchi *et al.* constructed an IRA in rats and measured villus length at 30 days post-op and did not find villous blunting.¹⁹ The current study sought to improve upon these existing models and to our knowledge, is the first to demonstrate the presence of colonic metaplasia, evaluated by architectural changes (villous blunting), changes in the mucin content, and increased *Wnt5a* gene expression. We elected to compare the IRA site to healthy upstream small bowel rather than non-surgical controls to eliminate potential within-group variation due to differences in surgical factors (i.e. time, surgical differences, microbiome). This method also allowed us to directly identify the changes to the small bowel within the same rat rather than comparing the overall trend for each group. We also evaluated the feasibility of endoscopy and biopsy to assess for the presence of inflammation and/or the changes of metaplasia in a longitudinal

model without animal sacrifice. The use of colonoscopic animal models have been developed and in some cases, coupled with radiographic measures^{20, 21} to study chemically-induced colitis^{22–25} and colorectal tumors.²⁶ To our knowledge, using endoscopy to study a surgical model of IRA or IPAA has not been previously performed. Longitudinal evaluation using repetitive endoscopy and biopsy of IRA or IPAA would facilitate studies evaluating pathogenesis of intestinal metaplasia and its possible role in inflammation, such as that seen in pouchitis or after ileo-colectomy.

Several considerations should be taken when performing IRA surgery. IRA surgery can be successfully performed on Sprague-Dawley rats weighing at minimum 250 g and in general, the younger rats tended to recover better than the older rats. At 1 week post-op, the anastomotic site was too swollen to permit passage of the endoscope; however, at 2 weeks post-op the anastomosis was patent with minimal surgery-induced changes and allowed passage of stool. Since a total colectomy was performed, the presence of stool at the time of surgery did not influence surgical outcomes and therefore, a mechanical bowel preparation was not performed. In addition, the bowels were not prepped prior to endoscopic evaluation. Instead, a saline flush through the endoscope was used to remove stool that may have interfered with visualizing the anastomotic site and ileum.

Stucchi *et al.* previously described an IRA model where animals demonstrated significant weight loss during the first 10 days post-op. The present protocol offers some improvement. Rats with IRA only lost weight during the first 2–3 days after surgery. This difference may be attributed to the use of a liquid purified low-residue diet administered to rats for the first 10 days post-op compared to administration of only water containing 5% dextrose for 4–5 days post-op used previously.^{18, 19} The liquid purified low-residue diet provided the nutrients required for the rat to recover and gain weight post-op but still allowed proper healing of the anastomotic site. The low fiber content aided in minimizing stool bulk and reducing frequency. Administration of a liquid formulation kept the stool consistency soft and diarrheal, reducing pressure on the anastomosis. Additionally, the use of a handheld cautery during colectomy minimized blood loss and time required to perform the surgery. This is important because the longer the surgery time, the higher the risk for intra-operative fluid loss. Administration of Lactated Ringer's before and after surgery helped with fluid management and none of the rats required additional fluids post-operatively. This model has several similarities and advantages over creating an IPAA. Colonic metaplasia is demonstrated similar to that seen in the IPAA but with a less technically demanding operation and less overall morbidity. In addition, the return of normal stool pellets over time implies the ileum assumes somewhat of a reservoir function, comparable to an IPAA.

Colonic metaplasia is found in humans with IRA⁴ and IPAA.^{5, 27} Similar to other studies,^{18, 19} some villous blunting was seen in areas near the anastomosis at the study endpoint. Contrary to a previous report,¹⁷ we identified colonic metaplasia (both villous blunting and presence of sulfomucin) in 70% of rats at the study endpoint. In a study that constructed ileal self-filling loops in mice to evaluate stasis-induced inflammation, colonic metaplasia was seen, correlating with up-regulation of *Wnt5a*.¹⁴ Similar to this study, we found that *Wnt5a* expression was enhanced in rats with colonic metaplasia relative to those without after IRA surgery. Additionally, no endoscopic evidence of inflammation was seen

throughout the study course of 8 weeks. Therefore, our data indicate that the presence of colonic metaplasia develops prior to inflammation. Utilizing a longitudinal animal model will provide for a better understanding of how and why colonic metaplasia develops and its association to inflammation.

Conclusions

This study demonstrated that colonic metaplasia developed within 4–8 weeks in 70% of Sprague-Dawley rats following IRA surgery. The majority of rats demonstrated villous blunting, changes in the mucin content within the upstream ileum, and increased levels of *Wnt5a* gene expression. This model has the potential to study the mechanism of colonic metaplasia, and possibly inflammation, and could form the basis for more sophisticated surgical models, such as IPAA and chemically-induced pouchitis models.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Improved recovery of younger rats following IRA surgery. **(A)** The study design defines the surgical and endoscopic course over the 8 week study period. At day 0, colectomy with IRA was performed with administration of Boost™ overnight. Starting on day 1, a liquid purified low-residue diet was administered for 10 days. Endoscopy was performed biweekly starting at day 14 throughout the remainder of the study. **(B)** Shown is average percent weight change examined in younger rats ($n = 13$, <350 g at surgery, red line), older rats ($n = 4$, >350 g at surgery, blue line), and a non-operative control (green line). Error bars designate standard deviation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ comparing >350 g at surgery and <350 g at surgery.

Fig. 2.

Cellular evidence of colonic metaplasia is present 4 weeks post-op although endoscopic inflammation is absent. **(A)** The Olympus MAF-GM mobilescope used in endoscopy to visualize the ileorectal anastomosis. **(B)** Rectal insertion of the MAF-GM mobilescope in an anesthetized rat. **(C)** Endoscopy of a rat low ileorectal anastomosis model was performed. The suture line (closed arrowheads) was visualized and the scope was passed through the anastomosis to assess for inflammation. **(D)** Hematoxylin and eosin-stained biopsy tissue taken about 1 cm above the anastomosis at 4 weeks post-op demonstrates villous blunting. **(E)** High iron diamine-Alcian blue stain of an ileal biopsy at 4 weeks post-op indicates the presence of both colonic sulfomucin and small bowel sialomucin within the ileum.

Fig. 3.

Evidence of colonic metaplasia in the terminal ileum above the ileorectal anastomosis at 8 weeks. **(A)** Representative image of H&E stained tissue with the ileorectal anastomosis (IRA). The anastomosis (closed arrowhead) is surrounded by villous blunting and crypt hyperplasia (boxed area), indicative of severe colonic metaplasia. **(B)** Ileal tissue upstream (~1 cm) of the anastomotic site shown in **(A)** depicts the progression from colonic metaplasia (boxed area) to more normal ileal architecture. **(C)** Representative H&E stained tissue from the IRA (closed arrowhead) of a rat with minimal evidence of colonic metaplasia with villous blunting demonstrated immediately upstream (boxed area) of the anastomotic site. **(D)** Measurement of villus length at the anastomosis (red bars) and upstream at an area of normal ileal architecture in the same rat (blue bars). Student's *t*-test was performed to compare measurements within the same rat. **P* 0.05, ***P* 0.01, ****P* 0.001

Fig. 4. Colonic mucins in terminal ileum indicate colonic metaplasia at 8 weeks. **(A)** High iron diamine-Alcian blue (HID-AB) stain of the ileorectal anastomosis (IRA) (closed arrowhead) from a rat that developed colonic metaplasia. The purple sulfomucin starts to develop upstream of the anastomotic site. Right; higher magnification of boxed area. **(B)** HID-AB stain of the IRA (closed arrowhead) from a rat without evidence of mucin alteration after 8 weeks. The blue sialomucin and purple sulfomucin delineate the anastomotic site. Right; higher magnification of boxed area.

Fig. 5.

Heightened *Wnt5a* gene expression in the terminal ileum correlates with development of colonic metaplasia defined by histology and changes in mucin content. RT-qPCR analysis of *Wnt5a* from terminal ileum collected at the time of surgery and dissected from the anastomosis at study endpoint. Shown are relative *Wnt5a* levels in the terminal ileum at the time of surgery (blue bars) and levels at study endpoint (red bars).

Summary of colonic metaplasia measurements.

Table 1

Rat	Villus length of normal ileum (mm)	Villus length at anastomosis (mm)	Mucin content of ileum upstream of anastomosis	Relative <i>Wnt5a</i> expression at surgery	Relative <i>Wnt5a</i> expression at 8 weeks
1	0.42 ± 0.03	0.35 ± 0.04**	Mixed	++	++
4	0.44 ± 0.02	0.32 ± 0.05***	Mixed	+	+++
5	0.43 ± 0.01	0.38 ± 0.04**	Mixed	+	+++
6	0.41 ± 0.02	0.36 ± 0.05*	Mixed	++	+++
8	0.31 ± 0.02	0.22 ± 0.01***	Mixed	N/A	N/A
9	0.41 ± 0.02	0.31 ± 0.05**	Mixed	+	+++
10	0.41 ± 0.03	0.29 ± 0.06***	Mixed	+++	+++
2	0.41 ± 0.02	0.41 ± 0.09	Sialomucin	N/A	N/A
3	0.45 ± 0.04	0.41 ± 0.07	Sialomucin	+	+
7	0.35 ± 0.02	0.31 ± 0.04**	Sialomucin	+	++

Measurement of villus length compared between an area of normal ileal architecture and at the anastomosis within each rat by Student's *t*-test. The area of normal ileal architecture was measured as far away as possible from the anastomosis, at least 2 cm, where the ileum demonstrated similarly sized and shaped villi with normal architecture. N/A, RNA was unable to be isolated from tissue sample.

* *P* 0.05,

** *P* 0.01,

*** *P* 0.001 compared to matched villus length of normal ileum.