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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Columnar-Lined Esophagus Develops via Wound Repair in a Surgical Model of Reflux Esophagitis

Q27 Agoston T. Agoston,[1,*] Thai H. Pham,[2,*] Robert D. Odze,[1] David H. Wang,[3,4] Kiron M. Das,[5] Stuart J. Spechler,[6] and Rhonda F. Souza[6]

[1]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts; [2]Department of Surgery, [3]Department of Medicine, VA North Texas Health Care System, [4]Harold C. Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, Dallas, Texas; [5]Division of Gastroenterology, Department of Medicine, Rutgers Robert Wood Johnson Medical School, New Brunswick, New Jersey; [6]Department of Medicine, Center for Esophageal Diseases, Baylor University Medical Center, Center for Esophageal Research, Baylor Scott and White Research Institute, Q1 Dallas, Texas

SUMMARY

After esophagojejunostomy, rodents develop ulcerative esophagitis and a columnar esophageal lining widely assumed to develop from progenitor cell reprogramming. This analysis study of early events in this process shows that this metaplastic, columnar-lined esophagus develops via wound healing rather than epithelial reprogramming.

BACKGROUND & AIMS: After esophagojejunostomy, rodents Q5 develop reflux esophagitis and a columnar-lined esophagus with features of Barrett's metaplasia. This rodent columnar-lined esophagus has been proposed to develop from cellular reprogramming of progenitor cells, but studies on early columnar-lined esophagus development are lacking. We performed a systematic, histologic, and immunophenotypic analysis of columnar-lined esophagus development in rats after esophagojejunostomy.

METHODS: At various times after esophagojejunostomy in 52 rats, the esophagus was removed and tissue sections were evaluated for type, location, and length of columnar lining. Molecular characteristics were evaluated by immunohistochemistry and immunofluorescence.

RESULTS: At week 2, ulceration was seen in esophageal squamous epithelium, starting distally at the esophagojejunostomy anastomosis. Re-epithelialization of the distal ulcer segment occurred via proliferation and expansion of immature-appearing glands budding directly off jejunal crypts, characteristic of wound healing. The columnar-lined esophagus's immunoprofile was similar to jejunal crypt epithelium, and columnar-lined esophagus length increased significantly from 0.15 mm (±0.1 SEM) at 2 weeks to 5.22 mm (±0.37) at 32 weeks. Neoglands were found within esophageal ulcer beds, and spindle-shaped cells expressing epithelial-mesenchymal transition markers were found at the columnar-lined esophagus's leading edge. Only proliferative squamous epithelium was found at the proximal ulcer border.

CONCLUSIONS: After esophagojejunostomy in rats, metaplastic columnar-lined esophagus develops via a wound healing process that does not appear to involve cellular reprogramming of progenitor cells. This process involves EMT-associated migration of jejunal cells into the esophagus, where they likely have a competitive advantage over squamous cells in the setting of ongoing gastroesophageal reflux disease. *(Cell Mol Gastroenterol Hepatol 2018;■:■–■; https://doi.org/10.1016/j.jcmgh.2018.06.007)*

Keywords: Gastroesophageal Reflux; Barrett's Esophagus; Epithelial-Mesenchymal Transition.

Barrett's esophagus, the condition in which an Q7 abnormal columnar mucosa with both gastric and intestinal features replaces esophageal squamous mucosa damaged by gastroesophageal reflux disease (GERD),[1,2] is a Q8 major risk factor for esophageal adenocarcinoma.[3,4] The pathogenesis of Barrett's esophagus is judged to involve Q6 GERD-induced alterations of key developmental transcription factors expressed by the cells that give rise to "metaplastic" columnar mucosa. The identity of those Barrett's progenitor cells is not known, but a number of potential candidates recently were reported (reviewed by Wang and Souza[5]). It has been proposed that GERD might cause mature esophageal squamous cells to transdifferentiate into columnar cells, or cause immature esophageal progenitor cells (in the basal layer of the squamous epithelium or in the ducts of esophageal submucosal glands) to undergo columnar rather than squamous differentiation (a process known as transcommitment) (reviewed by Wang and Souza[5]). Some investigators have suggested that Barrett's metaplasia might result from upward migration of stem cells from the gastric cardia,[6] or from proximal expansion of unique populations of residual embryonic

*Authors share co-first authorship.

Abbreviations used in this paper: GERD, gastroesophageal reflux disease; Dcamkl1, doublecortin and CaM kinase-like-1; EMT, epithelial-mesenchymal transition; Msi-1, Musashi-1; Muc, mucin; Pdx1, pancreatic and duodenal homeobox 1; Sox, sex determining region Y-box.

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cells[7] or transitional basal cells[8] located at the squamocolumnar junction. Other investigators have proposed that circulating bone marrow stem cells that settle in the reflux-damaged esophagus are the Barrett's progenitors.[9]

Animal models, primarily involving rodents, have been used to explore the pathogenesis of Barrett's esophagus. In 1962, Levrat et al[10] reported that they could induce severe ulcerative reflux esophagitis in rats by connecting the duodenum to the esophagus (ie, creating an esophagoduodenostomy). Later, other investigators showed that some rats with reflux esophagitis induced by esophagoduodenostomy or by esophagojejunostomy developed a Barrett's-like columnar lining in the esophagus that was capable of neoplastic progression to dysplasia and adenocarcinoma.[11–13] Although many investigators since then have used esophagojejunostomy in rodents as a model for studying Barrett's metaplasia and its neoplastic progression, Q10 none systematically explored the early events whereby reflux esophagitis ultimately results in the development of a columnar-lined esophagus.

In our study, we conducted a systematic investigation of the early histologic events in the development of a columnar-lined esophagus in rats after esophagojejunostomy. These rats developed ulceration in the squamous-lined distal esophagus starting at the anastomotic site and progressing proximally up the esophagus, with subsequent progressive re-epithelialization of the distal portion of the ulcer bed (adjacent to jejunum) by an intestinal type of columnar epithelium. We used immunohistochemical techniques to characterize the native epithelia both proximal and distal to esophageal ulcers (squamous epithelium proximally, jejunal epithelium distally), as well as the cells that appeared to be re-epithelializing the proximal and distal portions of the ulcer bed. We found that the columnar-lined esophagus in this animal model develops via a wound healing process that includes epithelial-mesenchymal transition (EMT) of the intestinal cells at the ulcer edge, and not via genetic reprogramming of progenitor cells. We also found that the squamous epithelium at the proximal ulcer border showed decreased expression of a squamous transcription factor (sex determining region Y-box [Sox]2) while showing increased expression of a columnar transcription factor (Sox9), consistent with the beginning of a metaplastic process, but the cells still maintained their histologic squamous phenotype.

Q11 Materials and Methods

Animals

We used 78 Sprague–Dawley rats (age, 6 wk; average body weight, 250 ± 22 g; Charles River Laboratory, Wilmington, MA) for these studies. The animals were kept in conventional housing (12-hour light/dark cycle; ambient temperature, 72°F), and were fed standard rat chow with water given ad libitum; rat chow was withheld 1 day before surgery. Rats were anesthetized with intraperitoneal injections of ketamine (75 mg/kg) and xylazine (12 mg/kg). After surgery, rats were weighed and their condition was assessed daily for 5 days, then once every 2 weeks. Animals were euthanized by carbon dioxide asphyxiation with bilateral thoracotomies if they became ill or lost more than 20% of their preoperative body weight. The study protocol was approved by the Dallas Veteran Affairs Medical Center Institutional Animal Care and Use Committee.

Rat Model of Reflux Esophagitis (Esophagojejunostomy)

Gastroesophageal reflux was induced by fashioning an esophagojejunostomy as previously described by Levrat et al[10] in 60 animals. In brief, using a small upper midline laparotomy, the stomach was mobilized and the gastroesophageal junction was ligated with 5-O polypropylene Q12 suture. The esophagus was divided just proximal to the suture, an end-to-side esophagojejunal anastomosis was performed using 7-O polypropylene suture, and the laparotomy incision was closed using 5-O polypropylene suture. For controls, we performed a sham surgery comprising anesthesia, celiotomy, and dissection of the gastroesophageal junction without esophageal transection or esophagojejunostomy. Animals were fed a liquid diet of Ensure (Abbott Nutrition, Columbus, OH) for 5 days after surgery, and then restarted on standard rat chow. The preoperative and postoperative care of the esophagojejunostomy and sham-operated control rats was identical.

The overall mortality rate for the esophagojejunal anastomosis group was 13%. Deaths resulted from aspiration (5 animals), bowel obstruction from ingestion of a foreign body (2 animals), and evisceration in the immediate postoperative period (1 animal), leaving 52 animals with an esophagojejunal anastomosis available for study. There were no deaths in the sham-operated group, leaving 18 control animals available for study.

Tissue Handling and Pathologic Evaluation

Groups of at least 5 animals (total, 60 rats) that had esophagojejunostomy were euthanized at postoperative weeks 2, 4, 6, 8, 10, 12, 16, 24, and 32; 2 sham-operated control rats were euthanized at each corresponding time point. The esophagus, including the esophagojejunal anastomosis, was removed en bloc and opened longitudinally. Sample strips of esophagus and jejunum were snap frozen and stored in liquid nitrogen. The remaining esophagus was pinned flat on a corkboard and fixed in 10% buffered formalin for 24 hours. The esophagus was cut into 2-mm–wide sections, which were oriented and placed into cassettes before paraffin fixation. Serial sections (4-μm–thick) were mounted on glass slides and stained with H&E for histologic evaluation. Two gastrointestinal pathologists (R.D.O. and A.T.A.), who were blinded to surgical group, evaluated the esophageal specimens for a variety of histologic features in the squamous epithelium, ulcer base, and columnar epithelium at the esophagojejunal anastomosis. The squamous epithelium was evaluated for the presence and degree of esophagitis, surface erosion or ulceration, and type and degree of inflammatory infiltrate. Specific histologic features such as the degree of basal cell hyperplasia, papillary hyperplasia, and intercellular edema