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ORIGINAL RESEARCH

The Development of Spasmolytic Polypeptide/TFF2-Expressing Metaplasia (SPEM) During Gastric Repair Is Absent in the Aged Stomach

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SUMMARY

This study demonstrates the emergence of spasmolytic polypeptide/TFF2-expressing metaplasia during regeneration of the gastric epithelium. The development of spasmolytic polypeptide/TFF2-expressing metaplasia in response to injury is absent in the aged stomach. In addition, transplantation of gastric organoids promoted gastric regeneration.

BACKGROUND & AIMS: During aging, physiological changes in the stomach result in more tenuous gastric tissue that is less capable of repairing injury, leading to increased susceptibility to chronic ulceration. Spasmolytic polypeptide/trefoil factor 2–expressing metaplasia (SPEM) is known to emerge after parietal cell loss and during *Helicobacter pylori* infection, however, its role in gastric ulcer repair is unknown. Therefore, we sought to investigate if SPEM plays a role in epithelial regeneration.

METHODS: Acetic acid ulcers were induced in young (2–3 mo) and aged (18–24 mo) C57BL/6 mice to determine the quality of ulcer repair with advancing age. Yellow chameleon 3.0 mice were used to generate yellow fluorescent protein–expressing organoids for transplantation. Yellow fluorescent protein–positive gastric organoids were transplanted into the submucosa and lumen of the stomach immediately after ulcer induction. Gastric tissue was collected and analyzed to determine the engraftment of organoid-derived cells within the regenerating epithelium.

RESULTS: Wound healing in young mice coincided with the emergence of SPEM within the ulcerated region, a response that was absent in the aged stomach. Although aged mice showed less metaplasia surrounding the ulcerated tissue, organoid-transplanted aged mice showed regenerated gastric glands containing organoid-derived cells. Organoid transplantation in the aged mice led to the emergence of SPEM and gastric regeneration.

CONCLUSIONS: These data show the development of SPEM during gastric repair in response to injury that is absent in the aged stomach. In addition, gastric organoids in an injury/transplantation mouse model promoted gastric regeneration. *(Cell Mol Gastroenterol Hepatol 2016;2:605–624; http://dx.doi.org/10.1016/j.jcmgh.2016.05.004)*

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During aging, changes in the stomach result in gastric tissue that is less capable of repairing injury correctly. These changes include decreased gastric acid secretion, motility, and proliferation.[1] In addition, angiogenesis, a fundamental process essential for wound healing, is impaired with advanced age.[2–4] Such pathophysiological changes are believed to result in disrupted repair in response to chronic ulceration in the elderly that can be exacerbated during chronic insults such as *Helicobacter pylori* infection or nonsteroidal anti-inflammatory drug administration.[5] In elderly patients there is a strong

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Abbreviations used in this paper: **CD44v, variant isoform of CD44; Cftr, cystic fibrosis transmembrane conductance regulator; CgA, chromagranin A; Clu, Clusterin; Ctss, cathepsin S; Dmbt1, deleted in malignant brain tumors 1; DMEM, Dulbecco's modified Eagle medium; DPBS, Dulbecco's phosphate buffered saline; ES, enrichment score; Gpx2, glutathione peroxidase 2 (gastrointestinal); GSEA, gene set enrichment analysis; GSII, *Griffonia simplicifolia II*; hFGO, human-derived fundic gastric organoid; HK, hydrogen potassium adenosine triphosphatase; IF, intrinsic factor; Mad2l1, MAD2 mitotic arrest deficient-like 1; Mmp12, matrix metallopeptidase 12 (macrophage elastase); PBS, phosphate-buffered saline; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; SPEM, spasmolytic polypeptide expressing metaplasia; TFF, trefoil factor; TX, Triton X-100 in PBS; UEA1, ulex europaeus; Wfdc2, WAP 4-disulfide core domain 2; YFP, yellow fluorescent protein.**

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association between ulceration with cancer or evolution of dysplasia into neoplasia.[6] Renewal of gastric stem cells to produce committed progenitor cells that differentiate further into adult epithelial cell types is important for the structural integrity of the mucosa. However, relatively little is known regarding the age-related changes affecting gastric epithelial stem cells. Early studies have shown that in aged rats, stem cell proliferation and epithelial cell numbers are decreased compared with young animals,[7] thus suggesting impaired tissue integrity in the aged stomach.

The origin of cells for repair of severe gastric epithelial injury has not received extensive attention. Recent investigations have indicated that loss of parietal cells, either from acute toxic injury or chronic *Helicobacter* infection, leads to the development of spasmolytic polypeptide/trefoil factor (TFF) 2–expressing metaplasia (SPEM) through transdifferentiation of chief cells into mucous cell metaplasia.[8,9] In the face of continued inflammation and M2-macrophage influence, SPEM may progress to a more proliferative preneoplastic metaplasia.[10] However, studies with acute injury have indicated that SPEM disappears after resolution of injury.[11] Whether SPEM may contribute to the healing of gastric ulcers is unknown. We now report that SPEM represents a major reparative lineage responsible for wound healing after gastric ulcer injury. In addition, the healing of gastric ulcers in the aged stomach is promoted by the transplantation of gastric organoids.

Materials and Methods

Mouse-Derived Gastric Organoid Culture

Gastric organoids were generated as previously described.[12–14] Yellow chameleon 3.0 mice were used to generate yellow fluorescent protein (YFP)-expressing organoids for transplantation. Briefly, the stomach was opened along the greater curvature and washed in phosphate-buffered saline (PBS). A dissecting microscope was used to remove the muscle layer. The remaining tissue was cut into pieces smaller than 5 mm^2 and incubated in 5 mmol/L EDTA in Dulbecco's phosphate buffered saline (DPBS) (without Ca^{2+} and Mg^{2+}) for 2 hours on a shaker at 4°C. For gastric gland dissociation 5 mL of dissociation buffer (55 mmol/L D-sorbitol and 43 mmol/L sucrose in DPBS without Ca^{2+} and Mg^{2+}) was added to tissue and vigorously shaken for 2 minutes. Media containing glands was centrifuged at 65 × g for 5 minutes. Glands were resuspended in Matrigel Corning Incorporated (Tewksbury, MA) and 50 μL of glands suspended in Matrigel was added to each well. Gastric organoid media containing 50% Wnt conditioned media, 10% R-spondin conditioned media, [Leu15]-gastrin 1 (10 nmol/L; Tocris, Pittsburgh, PA), N-acetylcysteine (1 mmol/L; Sigma, St. Louis, MO), fibroblast growth factor 10 (100 ng/mL; PeproTech, Rocky Hill, NJ), epidermal growth factor (50 ng/mL; PeproTech), Noggin (100 ng/mL; PeproTech), Y-27632 (10 μmol/L; Sigma), and advanced Dulbecco's modified Eagle medium (DMEM)/F12 was added after Matrigel polymerization at 37°C. Organoids were cultured 7 days before transplantation. The L cells were a kind gift from Drs Meritxell Huch, Sina Bartfeld, and Hans Clevers (Hubrecht Institute for Developmental Biology and Stem Cell Research, The Netherlands). The modified human embryonic kidney–293T cells were donated by Dr Jeffrey Whitsett (Section of Neonatology, Perinatal and Pulmonary Biology, Cincinnati Children's Hospital Medical Center and The University of Cincinnati College of Medicine, Cincinnati, OH).

Human-Derived Gastric Organoid Culture

Gastric organoids were generated as previously described.[15] Human fundic gastric tissue was obtained from Cincinnati Children's Hospital with patient consent. Patients were aged between 15 and 19 years. Human fundus was collected during sleeve gastrectomies (Institutional Review Board protocol number: 2013-2251). The human gastric tissue was cut into pieces smaller than 5 mm^2 before being suspended in advanced DMEM/F12 supplemented with 20 mmol/L HEPES and 2 mmol/L Glutamax (Thermo Fisher Scientific). A total of 1 mg/mL collagenase (C9891; Sigma) and 2 mg/mL bovine serum albumin were added to the supplemented advanced DMEM/F12 media. The solution was placed on a stir plate in a water bath at 37°C with oxygen for 30 minutes. The tissue was strained through mesh to collect the gastric glands and remove any undigested tissue. Gastric glands were washed with DPBS (without Ca^{2+} and Mg^{2+}) containing antibiotics. Isolated gastric glands were embedded in Matrigel and overlayed with organoid growth media containing 30% advanced DMEM/F12, 10 mmol/L HEPES, 2 mmol/L Glutamax, 1× N2, 1× B27, 1 mmol/L N-acetylcysteine, 10 mmol/L nicotinamide, 50 ng/mL epidermal growth factor, 100 μg/mL Noggin, 10% R-spondin conditioned media, 50% Wnt conditioned media, 100 μg/mL fibroblast growth factor 10, 1 nmol/L gastrin, 1% penicillin/streptomycin, 50 mg/mL kanamycin, 0.25 mg/mL amphotericin B, 10 mg/mL gentamicin, and 10 μmol/L Y-27632.

Acetic Acid–Induced Gastric Injury and Organoid Transplantation

All mouse studies were approved by the University of Cincinnati Institutional Animal Care and Use Committee, which maintains an American Association of Assessment and Accreditation of Laboratory Animal Care facility. Young mice (age, 2–3 mo) and aged mice (age, >18 mo) (C57BL/6) were subjected to acetic acid gastric injury as previously described.[16] Briefly, mice were anesthetized with isoflurane. The stomach was exteriorized through a midline abdominal laparotomy opening. One hundred percent acetic acid was applied to the serosal surface of the exteriorized stomach for 25 seconds using a capillary tube. Organoids were transplanted at the same injury site. Before transplantation, organoids were washed twice with ice-cold DPBS (without Ca^{2+} and Mg^{2+}) to remove Matrigel. Organoids then were resuspended in DPBS to a concentration of approximately 500 organoids per 50 μL. Immediately after ulcer induction either 50 μL DPBS or 50 μL organoids were injected into the muscle and submucosa of the stomach surrounding the ulcer site using a 26G × 3/8 syringe (309625; Thermo Fisher, Waltham, MA). The stomach then was replaced into the