METHODS

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Characteristics of human tissue samples

Entire stomachs were obtained from three non-obese individuals at the time of organ harvest under an IRB-approved protocol that was designated as non-human subject research. The characteristics of the subjects are detailed in Supplemental Table 1. All three donor stomach samples were negative for *H. pylori* by CLO test on antral mucosa. Donor specimens were opened along the greater curvature of the stomach, fixed for a minimum of 10 hours in 10% buffered formalin, and submitted for standard processing for FFPE tissue. Each specimen was dissected into 3 mm rows that were further divided into 2 cm width length segments. These segments were mapped onto the original specimen to allow for precise localization. Each block of stomach tissue was examined with H&E stain for orientation of the mucosal glands. Areas of mucosa with optimal orientation from each block were selected for 1 mm core excision and arraying into tissue microarrays (TMA). A single tissue core was selected from block. TMAs were constructed for each donor stomach in the Vanderbilt Translational Pathology Shared Resource.

For correlative studies of antral gland anatomy, we used antral sections from six further non-obese human donors all of whom were *H. pylori* negative.

Immunostaining.

Human stomach sections were deparaffinized and submitted to antigen retrieval in a pressure cooker using the Target Retrieval solution (Dako North America, Inc., Carpinteria, CA). Primary antibody incubation was performed overnight at 4 ^{0}C. For immunohistochemistry, secondary antibody incubation and DAB development were performed using the Dako Envision+ System-HRP DAB according to the manufacturer's instructions. In the case of immunofluorescence, appropriate secondary antibodies conjugated with Alexa 488, Cy3, or Cy5 were used (1h incubation at room temperature).

In some cases, for dual labeling using two rabbit primary antibodies, we employed the TSA (Tyramide Signal Amplification)-Plus Fluorescein or Cyanine 3 Kits (PerkinElmer) to detect the first primary antibody of each pair (used in this case at a dilution 10-20 fold higher than in a regular staining). After washes with 1X PBS and a second blocking step, we performed the incubation with the second primary antibody of each pair, which was then detected using Alexa 488- or Cy3-conjugated secondary antibodies. In both cases, we included a control slide for which the second primary antibody of each pair was omitted. Detailed information on primary antibodies and their dilutions are listed in Supplemental Table 2.

Tissue array imaging and quantitation.

Immunostained tissue microarray slides were imaged on the Ariol SL-50 automated slide scanner (Leica). Slides were imaged at 20X magnification to a resolution of 0.323 µm/pixel. Cells were identified in each core by utilizing standard Ariol analysis scripts. Upper and lower thresholds for color, saturation, intensity, size, roundness, and axis length were set for both blue Hematoxylin staining of nuclei and for brown DAB reaction

products. Thus, brown (DAB) positive cells can be distinguished from blue (Hematoxylin only) negative cells. Cell numbers were recorded for each core and the data were imported into Microsoft Excel to aide in the creation of 3-dimensional maps of each staining pattern.

To analyze the distribution of cell lineages with the stomachs, we defined a zone in the distal stomach as antrum based on the distribution of gastrin cells. We then defined three zones in the remaining body region by dividing the distance of the remaining specimens in thirds based on the cephalo-caudal axis (see Figure 1). The percent of cores in each zone were as follows: Donor 1: Body 1, 24%; Body 2, 31%; Body 3, 27% and Antrum, 18%. Donor 2: Body 1, 27%; Body 2, 32%; Body 3, 26% and Antrum, 15%. Donor 3: Body 1, 29%; Body 2, 33%; Body 3, 25% and Antrum, 13%. For each of the labeled cell lineages the distribution was defined by determining the number of cells in each zone as a percent of the total number of labeled cells in the entire specimen. These percentages were then compared across all three specimens using an ANOVA with post-hoc comparison of significant means with Bonferroni's test ($p<0.05$ for significance).

3-Dimensional rendering of stomach staining.

The numbers of cores representing each stomach were so great that they needed to be spread over multiple slides. Thus, for each immunostain tested between 2 to 5 slides were analyzed for each stomach. Data obtained from the analysis of tissue microarray cores for each stomach and stained marker were pooled into master lists in Microsoft Excel. Separately, 2-dimentional maps were created in Adobe Illustrator CS6 to characterize the

original layout of each stomach and specifically identify the origin of each core sample. Following Ariol analysis, the numbers of positive cells identified in each core were translated onto the 2-dimentional maps in Adobe Illustrator. A “heat map” of color ranges from solid blue to solid red was created to represent the range of positive cells for each immunostained marker. Basically, the natural log of each cell count present in each stomach was listed in ascending order. This order was then assigned a value using the following equation where A equals the natural log of the cell count:

Hue = ROUND((240-(A2*(240/(MAX(OFFSET(A1,1,0,COUNTA(A:A),1))-MIN(OFFSET(A1,1,0,COUNTA(A:A),1)))))),0)

The values produced by this equation were then utilized to create HSB (Hue, Saturation, Brightness) color swatches where the Hue value was variable between 240 and 0, while the Saturation and Brightness values remained 100%. Each cell representing an individual cell count in each 2-dimentional map was colored with the HSB value corresponding to cell count (Supplemental Figures 1 and 2). Colored 2-dimentional stomach maps were then stretched into square Mercator projections of themselves in order to create image maps for 3-dimentional modeling. A wireframe general stomach model was created in the professional modeling software Cinema 4D (MAXON Computer GmbH, Germany) based on rough axis ratio measurements of human stomachs. The 2-dimentional Mercator projections for each stomach were then “wrapped” around this 3-dimentional wireframe model. Wrapped models were then rendered and animated in Cinema 4D to produce tiff images and Quicktime movies.

Three-dimensional reconstruction of human antral glands.

Thirteen serial 5 μm human stomach sections were cut from a gastric antrum block. Immunolabeling was performed using the same procedure as above in the immunohistochemistry protocol. Briefly, the Gastrin primary antibody (at a dilution 1:8,000) was incubated overnight at 4 ^{0}C, then the Gastrin primary antibody was detected using TSA (Tyramide Signal Amplification)-Plus Cyanine 3 Kit. After the second blocking step using Protein Block Serum-Free solution, the H/K-ATPase and P120 antibodies were incubated at 4 °C for overnight, which were then detected using Alexa448- and Cy5-conjugated secondary antibodies with DAPI staining of nuclei.

The serial sections were imaged with the Ariol SL-50 automated slide scanner at 20X magnification to a resolution of 0.323 μm/pixel. A matching, aligned, representative area was extracted from each of the serial sections and saved in Tagged Image File Format (TIFF). Individual stomach glands were traced out using in Adobe Photoshop CS6 (Adobe), using the anti-p120 and DAPI staining as guides. The traces were then reassembled into a 3-dimentional object using the Imaris software package (Bitplane/Andor Technology).