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Immunohistochemistry and high resolution microscopy of human gastric enteroendocrine cells

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1 Works for me dx.doi.org/10.17504/protocols.io.w3tfgnn

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ABSTRACT

Enteroendocrine cells are important regulators of gastrointestinal, digestive and metabolic function. Here we describe protocols for identifying, mapping and characterising these cells in the human gastric mucosa using immunohistochemistry and superresolution microscopy techniques.

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KEYWORDS

Oxyntic gland, Gastrointestinal hormones, Ghrelin, 5-Hydroxytryptamine, Somatostatin, Pancreastatin

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MATERIALS TEXT

MATERIALS

Mouse Anti-Calbindin Antibody RRID:AB_2314069 **Swant Catalog #18F**

Rabbit anti-5-HT antibody AB_572263 **Immunostar Catalog #20080**

Goat anti-5-HT antibody AB_572262 **Immunostar Catalog #20079**

Mouse anti Gastrin/CCK RRID: AB_2650429 **Contributed by users Catalog #28.2**

Chicken anti-Ghrelin antibody RRID: AB_2041392 **Abcam Catalog #Ab15861**

Rabbit anti-GLP1 antibody RRID: AB_2650430 **Contributed by users Catalog #8912**

Mouse anti-mast cell tryptase antibody - RRID:AB_2206479 **Millipore Catalog #MAB 1222**

Rabbit anti-Gastrin antibody - RRID: AB_2762851 **Contributed by users Catalog #8007**

Chicken anti-PYY antibody RRID: AB_1855196 **Sigma Aldrich Catalog #GW22771**

Rabbit anti-Calbindin antibody **Contributed by users Catalog #R202**

Rabbit anti-Ghrelin antibody RRID: AB_2767291 **Contributed by users Catalog #RY1601**

Addition antibodies (no supplier):
Rabbit anti-Gastrin 8007 (gift from Dr Jens Rehfeld) RRID:AB_2762851

DISCLAIMER:

1 Stomach regions were collected from six patients who were undergoing gastric sleeve surgery for obesity at the Renown Regional Medical Center, Reno, Nevada. Resections were of the full greater curvature (from the fundus to the antrum) from male and female patients between the ages of 48 and 60 who were non-diabetic. The tissue was placed in cold fixative (2% formaldehyde plus 0.2% picric acid in 0.1 M sodium phosphate buffer, pH 7.0) kept overnight at 4 °C. Tissues were then washed three times (10 min) with dimethyl sulfoxide (DMSO) and then three times (10 min) with PBS. Tissue samples were then transferred to PBS-azide and sent to the University of Melbourne. The tissue samples were placed in 50% PBS-sucrose-azide and 50% OCT mixture (Tissue Tek, Elkhart, IN, USA) for 24 h, before being trimmed, embedded in 100% OCT and frozen in isopentane cooled with liquid nitrogen.

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2 Sections of 12 µm thickness were cut, allowed to dry at room temperature for 1 h on microscope slides (SuperFrost Plus®; Grale Scientific, Victoria, Australia) and incubated with 10% normal horse serum plus 1% Triton X-100 in PBS for 30 min. Mixtures of primary antibodies (Table1) for double staining were then placed on the sections that were left at 4 °C overnight. The tissue was washed three times in PBS and incubated with appropriate secondary antibodies labelled with Alexa Fluor dyes for 1 h at room temperature. To help reduce background fluorescence, tissue was washed with PBS for 5 min and then incubated with a quenching buffer (5 mM $CuSO_4$, 50 mM ammonium acetate, pH 5) for 30 min at room temperature. Preparations were then washed three times with PBS for 10 min. Preparations were washed twice with distilled water for 5 min then incubated for 5 min with bisbenzimide (blue), diluted 10 µg/mL in dH_2O, to stain nuclei. Sections were then washed three times with distilled water before mounting with non-fluorescent mounting medium (Dako, Carpinteria, CA, USA). An absorption test was applied to test the specificity of ghrelin antibodies binding to cells in the human stomach. The diluted anti-ghrelin antibodies were equilibrated with human ghrelin peptide (100 nM to100 µM) for 24 h at 4 °C before being used for staining of tissue sections as above. There was a concentration-related reduction in the immunohistochemical localisation. The immunoreactivity using chicken anti-ghrelin 14481 was reduced with 100 nM and 1 µM peptide and was abolished with 10 µM and 100 µM. Immunoreactivity with rabbit anti-ghrelin RY1601 was reduced with 1 µM peptide and was abolished with 10 µM and 100 µM. For all secondary antisera used, sections that were incubated without primary antibodies were used to investigate background staining and autofluorescence. There was no indication of non-specific binding of the secondary antibodies.

3 Slides were examined and imaged using an Axio Imager Z1 microscope (Zeiss, Sydney, Australia) and a LSM 800 confocal microscope with Airyscan super-resolution analysis (Zeiss). Tile scans taken with a × 10 objective were used for cell counts; for the density analysis, the number of cells was divided by the total area of the mucosal section that was counted. Tile scans were exported to be analysed off-line using ImageJ software (imagej.nih.gov/ij/). Immunoreactive cells were quantified by counting 100–500 cells in sections from each part of the stomach. This analysis was repeated in tissues from three patients. × 40 and × 63 objectives were used for high-resolution analysis. High-resolution images were exported into CorelDraw (Corel, Ottawa, Canada) for final preparation of figures. For analysis of 3D stacks, the Imaris program 8.4.1 (Bitplane, Oxford Instruments, Abington, UK) was used and image rendering was applied where appropriate. To determine whether the ghrelin cells occur in clumps, three pieces of the fundus from three different patients were analysed using the Delaunay triangulation protocol in ImageJ. Ghrelin cells were circled manually and the Delaunay triangulation for neighbour analysis was applied for the selected region of interest.

4 Prism 5.0 (GraphPad Software, San Diego, CA, USA) was used to analyse data and present it as mean ± SEM. Differences were evaluated using two-way ANOVA with the Bonferroni post hoc test. A Pvalue < 0.05 was taken as significant.

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