Experimental Cell Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Experimental Cell Research

journal homepage: www.elsevier.com/locate/yexcr

Tuft cells: Distribution and connections with nerves and endocrine cells in mouse intestine

Xiaowen Cheng*, Ulrikke Voss, Eva Ekblad

Department of Experimental Medical Science, Unit of Neurogastroenterology, Lund University, Sölvegatan 19, BMC B11, SE-22184 Lund, Sweden

ARTICLE INFO

Keywords:
Tuft cells
CGRP
PYY
CCK
GLP-1
Enteric nervous system

ABSTRACT

Tuft cells are gastrointestinal (GI) sensory cells recognized by their characteristic shape and their microvilli "tuft". Aims of the present study were to elucidate their regional distribution and spatial connections with satiety associated endocrine cells and nerve fibers throughout the intestinal tract. C57BL/6 J mice were used in the experiments. The small intestine was divided into five segments, and the large intestine was kept undivided. The segments were coiled into "Swiss rolls". Numbers and topographic distribution of tuft cells and possible contacts with endocrine cells and nerve fibers were estimated in the different segments, using immunocytochemistry. Tuft cells were found throughout the intestines; the highest number was in proximal small intestine. Five percent of tuft cells were found in close proximity to cholecystokinin-immunoreactive (IR) endocrine cells and up to 10% were in contact with peptide YY- and glucagon-like peptide-1-IR endocrine cells. Sixty percent of tuft cells in the small intestine and 40% in the large intestine were found in contact with nerve fibers. Calcitonin gene-related peptide-IR fibers constituted one-third of the fiber-contacts in the small intestine and two-thirds in the large intestine. These observations highlight the possibility of tuft cells as modulators of GI activities in response to luminal signaling.

1. Introduction

Food triggers sensations in the gastrointestinal (GI) tract. Sensory cells in the GI epithelium (e.g. tuft and endocrine cells) are able to detect luminal signals including nutrients and harmful components. These signals are important in the adaptation and modulation of GI functions (reviewed by [1]).

Tuft cells (also known as brush and caveolated cells) were, in the GI tract, initially described in the stomach and duodenum of mice [2]. They are recognized by their characteristic fusiform shape and distinct apical "tuft" of microvilli extending into the lumen [3]. Recent studies estimate that tuft cells make up 0.4–2% of the intestinal epithelial lining, and that they are preferentially located in villi and crypt-villus junctions [3,4]. Due to their expression of taste cell specific GTP-binding protein, α-gustducin, and taste-related cation channel transient receptor potential channel 5 (TRMP5) [5], tuft cells are suggested to act as luminal sensory cells. They are shown to trigger interleukin (IL)-25 associated immune reactions leading to expulsion of pathogens [6–8] and to be involved in epithelial protection and regeneration [9,10]. High-resolution single-cell sorting has identified two types of tuft cells [11]; one type expressing genes related to neuronal development and the other genes related to immune regulation.

It is still unclear how tuft cells transfer sensory signals from the lumen to other intestinal cells such as endocrine cells and neurons. Tuft cells harbour an intracellular tubular network spanning from the base of its microvilli to the endoplasmic reticulum, through which molecules can be exchanged between the lumen and the cell [12]. They also crosstalk with neighboring cells through lateral projections reaching into the neighboring cells' nuclei [12]. Additionally, tuft cells release paracrine mediators including nitric oxide [13]. Thus, there are several putative modes by which tuft cells mediate and exchange information from the lumen to other epithelial as well as non-epithelial cells.

Chemo sensation involves activation of both endocrine cells and nerve terminals in the GI tract. The GI tract comprises the body's largest population of endocrine cells, often identified and described by their hormone signatures [14–16]. Several satiety and appetite regulating hormones, e.g. cholecystokinin (CCK), peptide YY (PYY) and glucagon-like peptide-1 (GLP-1), are secreted from endocrine cells in response to nutrient stimulation [15]. Nerve fibers, of both extrinsic or intrinsic origin, innervate all intestinal layers [17–20]. It is suggested that

Abbreviations: CCK, cholecystokinin; CGRP, calcitonin gene-related peptide; DAB, diaminobenzidine; DCLK1, doublecortin-like kinase 1; HTX, hematoxylin; IL, interleukin; IR, immunoreactive; GI, gastrointestinal; GLP-1, glucagon like peptide-1; LI, large intestine; PBS-T, PBS with 0.25% Triton X-100; PGP 9.5, protein gene product 9.5; PYY, peptide YY; SI, small intestine; SP, substance P; TRMP5, transient receptor potential channel 5

* Corresponding author.
E-mail addresses: xiaowen.cheng@med.lu.se (X. Cheng), ulrikke.voss@med.lu.se (U. Voss), eva.ekblad@med.lu.se (E. Ekblad).

https://doi.org/10.1016/j.yexcr.2018.05.011
Received 7 March 2018; Received in revised form 9 May 2018; Accepted 10 May 2018

chemosensory cells, such as tuft and endocrine cells, act as an interface between the lumen and nerve fibers [21]. Tuft cells have been described in close contact with nerve fibers in the duodenojejunal junction in human [22], in mouse duodenum [23] and in rat ileum [24]. This highlights the potential of tuft cell-to-nerve interaction and crosstalk.

In this study, antibodies against doublecortin-like kinase 1 (DCLK1) protein, a highly selective and robust marker for tuft cells [25], were used to estimate number and distribution of tuft cells in various segments of mouse intestine. Additionally, contacts between tuft cells and satiety associated endocrine cells containing CCK, PYY or GLP-1 as well as contacts between tuft cells and nerve terminals containing the pan-neuronal marker PGP 9.5 or belonging to the calcitonin gene-related peptide (CGRP)-containing neuronal subpopulation were mapped.

2. Material and methods

2.1. Animals

Thirteen mice (C56BL/6 J background, male, weight 20–25 g) were purchased from Janvier Labs, France. Before sacrifice, they were housed for 8–12 days in groups of 3–5 in single ventilated cages and kept on a 12 h light/12 h dark cycle, with standard chow (R36, Lactamin, SE) and water freely available.

2.2. Ethical statement

All procedures were approved by the regional Malmö/Lund committee for experimental animal ethics, Swedish Board of Agriculture (M95-15). Animals were used in accordance with European Community Council Directive (2010/63/EU) and the Swedish Animal Welfare Act (SFS 1988:534).

2.3. Tissue harvesting

Animals were deeply anesthetized with an intraperitoneal (0.4 ml/20 g) injection of Rompun (0.4 mg/ml Bayer, German) and Ketalar (6 mg/ml, Pfizer, USA). An incision was made along the abdominal midline and visceral organs exposed. The intestines were harvested prior to sacrifice by heart puncture. The small intestine was collected 2 cm distally from the pyloric sphincter to cecum, and the large intestine removed from cecum to rectum. The small intestine was divided equally into 5 segments, labeled SI 1–5 from proximal to distal, 5–6 cm each. The five segments from the small intestine (SI 1–5) and the undivided large intestinal (LI) segment were opened longitudinally along the mesenteric line, and its content washed out. They were then coiled into “Swiss roll” as previously described [26,27].

For paraffin sectioning, Swiss rolls (n = 7) were immersion fixed in 4% paraformaldehyde in 0.1 M phosphate buffer overnight at 4 °C, and rinsed in 70% ethanol three times, followed by dehydration, clearing and embedding into paraffin until processed for immunohistochemistry.

For cryo sectioning, Swiss rolls (n = 6) were immersion fixed in Stefaninis fixative (0.2% picric acid, 2% formaldehyde in 0.1 M phosphate buffer, pH 7.2) overnight at 4 °C, and rinsed three times in Tyrode's solution containing 10% sucrose, all at 4 °C. The Swiss rolls were then embedded in FSC 22® Clear (Leica Biosystems, SE), frozen at − 80 °C until processed for immunofluorescence.

2.4. Immunohistochemistry

To estimate number and topographic distribution of tuft cells, paraffin-embedded Swiss rolls were cut into 5 μm thick sections and mounted on microscopic slides. They were deparaffinized with xylene and rehydrated through graded series of ethanol. All slides were subjected to antigen retrieval by microwaving 2 × 8 min at 650 W in citrated acid buffer (0.01 M, pH 6). After cooling, slides were washed for 20 min in running tap water, 10 min in 3% hydrogen peroxide to block endogenous peroxidase activity, and 10 min in PBS with 0.25% Triton X-100 (PBS-T). Sections were incubated overnight in the primary antiserum, rabbit anti-DCLK1 (1:250 dilution, code ab37994, Abcam [25,28,29]), at 4 °C. Sections were then rinsed in PBS-T buffer 3 × 10 min and incubated for 1 h in anti-rabbit IgG ImmPRESS reagent (#MP-7451, Vector laboratories, USA). Sections were then rinsed and incubated in 3,3′-diaminobenzidine (DAB) peroxidase (HRP) staining (#SK4100, Vector laboratories, USA) for 10 min. After rinse 3 × 10 min in PBS-T, sections were counter-stained in Hematoxylin (HTX), extensively washed and mounted in PERTEX® synthetic mounting medium (Histolab Products AB, Sweden).

2.5. Immunofluorescence

Cryo sections, 10 μm (for estimations on cell-to-cell contacts) or 20 μm (for estimations on cell-to-nerve contacts) thick were cut, mounted on microscopic slides and washed in PBS-T 10 min. To estimate possible contacts between tuft cells and endocrine cells or enteric nerve fibers, sections were processed for double-labeling using rabbit anti-DCLK1 combined with previously characterized antibodies raised in rabbit against PGP 9.5 (1:1600 dilution, code RA95101, Ultraclone, Isle of Wight, UK Isle of Wight, UK [30]), or CGRP (1:4800, code 8724, Euro-Diagnostica AB, Sweden, [31]), or in guinea pig against CCK (1:2000. Code 4468, kind gift from Professor J Rehfeld, Rigshospitalet, Copenhagen University, Denmark [32]), or PYY (1:2400. Code 8704, Euro-Diagnostica AB, Sweden, [33]), or in goat against GLP-1 (1:250, sc7782, Santa Cruz [34,35]). Slides were washed 3 × 10 min in PBS-T before incubation with secondary antibodies raised in donkey against rabbit IgG (Alexa Fluor594 conjugated, 711-585-152, 1:1500 Jackson Immuno Research, SE), guinea pig IgG (Alexa Fluor488 conjugated, 706-485-148, 1:1500 Jackson Immuno Research, SE) or goat IgG (Alexa Fluor488 conjugated, 705-545-147, 1:1200 Jackson Immuno Research, SE) overnight at 4 °C. After washing in PBS-T 3 × 10 min, sections were mounted in phosphate buffer:glycerol 1:1.

Antisera absorbed with excess of the respective peptide (10–100 μg/ml antiserum in working dilution) were used as controls. The CCK antiserum 4468 is C-terminally directed; consequently, it cross reacts with both CCK and gastrin and therefore cells labeled with antiserum 4468 should be referred to as gastrin/ CCK- immunoreactive (IR). Based on previous examinations on the distribution of gastrin- vs CCK-expressing cells [32,36] we, for simplicity, refer to them as CCK-IR in the following. Synthetic immunogens to the DCLK1 and the PGP 9.5 antisera are not commercially available therefore omission of primary antibodies was used as control.

2.6. Tuft cell estimation

Slides were scanned in bright-field setting at 40× using a digital slide scanner, Nanozoomer-2HT, and NDP2-viewer software (Hamamatsu, JP). The numbers of tuft cells in the various segments were estimated by cell counting and expressed as DCLK1-IR cells per villus, large intestinal surface epithelia intermediate to two adjacent crypts (in the following denoted as surface epithelia), crypt or together as crypt-villus unit, alternatively crypt-surface epithelia unit (crypt-villus/surface epithelia unit). The numbers of tuft cells were also estimated in relation to total cells in epithelium. At least 50 villi and crypts were included per intestinal segment. The possible correlation of the tuft cells in crypts and in villi was estimated by Pearson correlation coefficient (r).

2.7. Tuft cell contacts with endocrine cells and nerve fibers

Immunofluorescent slides were scanned in fluorescent mode on a digital slide scanner, Nanozoomer 2HT, equipped with appropriate filter settings and NDP2-viewer software (Hamamatsu, JP). Possible