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Enterochromaffin Cell–Enriched Monolayer Platform for Assaying Serotonin Release from Human Primary Intestinal Cells

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Abstract

Enteroendocrine (EE) cells within the intestinal epithelium produce a range of hormones that have key roles in modulating satiety and feeding behavior in humans. The regulation of hormone release from EE cells as a potential therapeutic strategy to treat metabolic disorders is highly sought after by the pharmaceutical industry. However, functional studies are limited by the scarcity of EE cells (or surrogates) in both *in vivo* and *in vitro* systems. Enterochromaffin (EC) cells are a subtype of EE cell that produce serotonin (5HT). Here, we explored simple strategies to enrich EC cells in *in vitro* monolayer systems derived from human primary intestinal stem cells. During differentiation of the monolayers, the EC cell lineage was significantly altered by both the culture method (air-liquid interface [ALI] *vs.* submerged) and the presence of vasoactive intestinal peptide (VIP). Compared with traditional submerged cultures without VIP, VIP-assisted ALI culture significantly boosted the number of EC cells and their 5HT secretion by up to 430% and 390%, respectively. The method also increased the numbers of other subtypes of EE cells such as L cells. Additionally, this method generated monolayers with enhanced barrier integrity so that directional (basal or apical) 5HT secretion was measurable. For all donor tissue, the enriched EC cells improved the signal-to-background ratio and reliability of 5HT release assays. The enhancement in 5HT secretion behavior was consistent over time from a single donor, but significant variation in the amount of secreted 5HT was present among tissues derived from 5 different donors. To demonstrate the utility of the EC-enriched monolayer system, 13 types of pungent food ingredients were screened for their ability to stimulate 5HT secretion. Curcumin found in the spice turmeric derived from the *Curcuma longa* plant was found to be the most potent secretagogue. This EC-enriched cell-monolayer platform can provide a valuable analytical tool for high-throughput screening of nutrients and gut microbial components that alter the secretion of 5HT.

Graphical Abstract

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Supporting Information

Formulation of culture media. List of suppliers, catalog numbers, stock solution concentrations, and storage conditions of reagents. Expansion and maintenance of human colonic epithelial stem cells on collagen hydrogel scaffolds. Effect of medium components on the formation of EC cells. Evaluation of 5HT secretion response per cell. Supplementary information Figure S1-S4.

INTRODUCTION

Dysregulated feeding behavior leads to excess eating and obesity. Obesity and its related metabolic impacts initiates a host of diseases that take a tremendous toll on human health and productivity. It is widely believed that hormones produced by enteroendocrine (EE) cells within the intestinal epithelium play key roles in control of feeding behaviors.¹ EE cells release hormones into the bloodstream in response to intestinal contents and other stimuli to modulate a variety of critical physiological functions including glucose homeostasis and food intake. A thorough understanding of the regulation and release of hormones from EE cells is necessary for implementing therapeutic approaches to treat obesity. There is thus a compelling interest in the biotechnology community for improved *in vitro* models of the human gastrointestinal system that enable meaningful and quantitative measurement of hormones produced by EE cells.

EE cells consist of at least 15 different subtypes with enterochromaffin (EC) cells being the most abundant, comprising ~40% of EE cells in the gastrointestinal tract.^{2,3} EC cells can sense various stimuli from luminal contents (*e.g.* nutrients, microbial metabolites, irritants, toxins, infection, and mechanical stimuli), and synthesize and secrete the biogenic amine 5-hydroxy tryptamine (5HT) also known as serotonin.^{4,5} More than 90% of the human body's total 5HT is located in the EC cells of the intestine, where it regulates gastrointestinal motility and secretion of digestive enzymes, as well as having critical roles in satiety and hunger suppression.⁵ Although EC cells play a crucial role in human physiology, they only constitute <1% of the human intestinal epithelial population. Their scarcity has posed a barrier to in-depth study of their sensory function and 5HT secretion. Tumor cell lines have not proven to be suitable mimics of intestinal EC cell physiologic responses. To try and overcome this challenge, live EC cells have been isolated from intestinal specimens, enriched by fluorescent activated cell sorting (FACS), and maintained in short-term culture for *in vitro* 5HT secretion and signaling studies.^{6,7} The main bottleneck of this approach, particularly for human studies, is the requirement for fresh tissue in each experiment as the cells are terminally differentiated and possess short (7 d) life spans. In the past decade, *in vitro* culture techniques of intestinal epithelial stem cells (IESCs) have made it possible to create primary cell-derived, physiologically relevant, *in vitro* intestinal models.^{8–10} These systems support the proliferation of IESCs by culturing them within (as organoids) or on top of (as monolayers) an extracellular matrix (ECM) in the presence of specific growth factors,

thus providing an unlimited supply of primary, human cells for *in vitro* studies.^{9,10} Monolayers of differentiated intestinal epithelial cells have been established by plating IESCs or IESCs-containing tissues (*e.g.* dissociated organoids) directly on an ECM-coated porous membrane followed by spontaneous or forced differentiation.^{10–16} The differentiated monolayers display characteristic polarized morphology and immunofluorescence markers of intestinal epithelium such as brush border proteins and tight junctions, and contain a diversity of intestinal epithelial cell lineages. EE and EC cells have been identified in these *in vitro* systems (organoids and monolayers) but their numbers are extremely low.^{11,12} The rarity of the EC cells within these culture systems severely limits their utility as analytical assay systems for the discovery of EC cell activators and inhibitors as the amount of hormone produced is at or below the limits of detection for many assays.

Several strategies have been developed to increase the presence of EE and EC cells in the *in vitro* systems. Co-culture of the epithelial monolayer with neurons and myofibroblasts was found to promote differentiation into EE cells increasing their fraction from 0.3% to 0.9%.¹⁷ In an organoid system, forced differentiation with combined EGFR/Wnt/MAPK inhibitors produced EE cells at high density (~50%).¹⁸ However, this inhibitor cocktail was quite toxic yielding extensive cell death with only small numbers of remaining viable cells. Recently, lentivirus transduction has been used to stably engineer IESCs with doxycycline-inducible expression of neurogenin-3 (NGN3), a transcription factor that drives EE differentiation.³ EE fraction increased from 0.4% (noninduced) to 40% (induced with doxycycline). Although this model is promising, the need to genetically engineer the cells presents disadvantages and limits the numbers of different genetic background that can be utilized.¹⁹

IESCs and their differentiated cells are generally maintained in a submerged or fully aqueous culture. Recently, air-liquid interface (ALI) culture was found to generate intestinal monolayers with improved secretory cell lineage differentiation.^{20–22} Our group modified the ALI culture system by adding an intestinal hormone, vasoactive intestinal peptide (VIP), to assist in the balance of fluid movement across the epithelium to hydrate the surface of the luminal tissue during differentiation.²¹ This VIP-assisted ALI culture method significantly boosted the formation of goblet cells and promoted mucus secretion and accumulation.²¹ Since both goblet and EE cells belong to the secretory cell lineage, it was likely that this approach might increase the presence of EE and EC cells in the *in vitro* monolayer system. In the current study, we assessed the impact of ALI and VIP on the presence of EE and EC cells in monolayers derived from human stem cells derived from the transverse colon, and studied 5HT secretion by applying forskolin, an activator of adenylate cyclase. The effect of passage number and donor identity on 5HT secretion was investigated. The utility of this model for screening was demonstrated by assaying the effect of 13 pungent food ingredients on 5HT secretion. The goal of this study was to establish a simple protocol to enrich EE and EC cells with only minor modifications of the current established monolayer protocol. The new protocol overcomes the limitations of other EE enrichment methods by maintaining high cell viability and barrier integrity without requiring complicated procedures of cocultures or genetic engineering/induction. This EC-enriched, contiguous monolayer platform is expected to act as a robust analytical tool to enable functional studies of 5HT secretion and inhibition from EC cells in the pursuit of finding dietary and pharmacologic interventions to modulate human eating behavior.

EXPERIMENTAL SECTION

Expansion and maintenance of human colonic epithelial stem cells.

IESCs were isolated and expanded from the transverse colon of cadaveric donor organs. The stem cells were maintained on 1-mm thick collagen hydrogel matrix in the 6-well plates using stem medium (SM): the process for expansion, maintenance, and passage of stem cells is described in the supplementary information using previously published protocols.^{10,23} Cells from one donor (female, 65 years old, donor 2) were used for all experiments except that in Figure 2I in which five donors were compared to study donor variation. The composition of media and the source of their ingredients are listed in supplementary information Table S1 and S2.

Generation of EC-enriched monolayer of human colonic epithelium on a porous substrate.

Transwell inserts possessing a porous membrane (0.4 μm pore size, either 24-well [Corning, 3470] or 96-well [Millipore, PSHT004S5]) were coated with 1 vol% Matrigel in phosphate-buffered saline (PBS) at 37°C overnight. The inserts were rinsed with PBS $\times 1$ prior to cell plating. The stem cells were then harvested from the expansion plate, passaged and suspended in expansion medium (EM) and plated directly onto the inserts. Cells from 1 well of the 6-well plate were dispersed into 16 separate 24-well inserts (0.2 mL in the upper [apical] reservoir, and 0.6 mL in the lower [basal] reservoir), or 48 separate 96-well inserts (0.1 mL in the apical reservoir, and 0.2 mL in the basal reservoir). The medium was exchanged after 48 h. After 4 days, to induce cell differentiation the medium was switched to differentiation medium (DM). In the submerged culture, DM was added in both apical and basal reservoirs (0.2 mL/0.6 mL upper/lower for 24-well inserts; 0.1 mL/0.2 mL for 96-well inserts). The medium was exchanged every 48 h thereafter. In the ALI culture, the medium in the apical reservoir was completely aspirated, DM or DM-VIP (DM containing 330 ng/mL vasoactive intestinal peptide [VIP, AnaSpec, AS-22872]) was added to the basal reservoir (0.6 mL for 24-well inserts; 0.2 mL for 96-well inserts), and medium at the basal reservoir was exchanged every 48 h thereafter. VIP stimulates the secretion of water onto the apical surface to hydrate the monolayer.^{21,24} By day 9, the monolayers (both submerged and ALI) were suitable for characterization and assays.

Imaging and quantification of EE and EC cells in colonic epithelial monolayers.

To reveal EE and EC phenotypes, monolayers in 24-well inserts were fixed with 4% paraformaldehyde for 15 min, permeabilized with 0.5% Triton-X 100 for 20 min, then blocked in 1% bovine serum albumin (BSA) for 1 h. The samples were subsequently co-stained with chromogranin A (Abcam, ab15160, rabbit, 1:1000) and 5HT (Novus Bio, NB120-16007, mouse 1:200) antibodies in 1% BSA for 16 h at 4°C, followed by staining with secondary antibodies (1:500) of goat anti-mouse (Alexa Fluor 488 conjugate, Jackson ImmunoResearch, 115-545-003) and goat anti-rabbit (Alexa Fluor 647 conjugate, Jackson ImmunoResearch, 111-605-003) for 1 h. Finally, nuclei were stained with Hoechst 33342 (2 $\mu\text{g/mL}$) for 15 min. The inserts were placed on a coverglass and the monolayers were imaged with an Olympus FluoView FV3000 confocal laser scanning microscope. The entire surface of inserts (surface area = 33 mm²) was scanned and imaged. The number of EE

(ChgA⁺) and EC (5HT⁺) cells were counted using Image J software. Three inserts were used for each condition to obtain the average cell density.

Permeability assay.

On the 96-well Transwell plate, the cells were rinsed with Hanks' balanced salt solution (HBSS, 150 μ L in the apical and 300 μ L in the basal reservoir) $\times$ 1. 75 μ L Lucifer yellow solution (LY, ThermoFisher, L11771, 190 μ M in HBSS) was added to the apical reservoir, and 250 μ L HBSS was added to the bottom. Samples were taken from the bottom at 3 h and assayed for fluorescence using a plate reader (SpectraMax M5, Molecular Devices). The LY concentrations were used to calculate the apparent permeability coefficient (P_{app}).

Stimulated secretion of 5HT and its quantification.

On the 96-well Transwell plate, the cells were rinsed with HBSS (150 μ L in the apical and 300 μ L in the basal reservoir) $\times$ 3 to remove 5HT residue from the culture media. The assay buffer was HBSS containing 1 μ M 5HT uptake inhibitor fluoxetine (Sigma, F132). Assay buffer (75 μ L) containing forskolin (10 μ M) or a dietary compound was added to the apical reservoirs, and 250 μ L assay buffer was added to the basal reservoirs. At 4 h, the supernatants from the basal reservoirs were collected for 5HT measurement. Thirteen dietary compounds were used in this study with their name, plant source and working concentrations listed in Figure 3C. The 5HT concentration was determined using an ELISA kit (Rocky Mountain Diagnostics, BA E-5900) according to the manufacturer's instruction. The samples were diluted 4 $\times$ so that all measured concentrations fell within the linear range (0.015 – 2.5 ng/mL) of the ELISA assay. Cell number (assessed by CellTiter-Glo luminescent cell viability assay) did not vary significantly between the different wells during each experiment. Unless otherwise specified, three samples were used for each condition. The change of 5HT concentration compared with vehicle control (DMSO, 1:1000) was analyzed statistically by two-tailed unpaired t-test. Changes were considered as statistically significant at $p < 0.05$ (denoted as *) and $p < 0.005$ (denoted as **).

RESULTS AND DISCUSSION

VIP-assisted ALI culture significantly increased the number of EE and EC cells, 5HT secretion, and barrier integrity.

The presence of EE (positive for chromogranin A, ChgA⁺) and EC (5HT⁺) cells was measured under standard culture conditions with two modifications. Nicotinamide, p38 MAP kinase inhibitor (SB202190) and valproate, three compounds commonly used in intestinal epithelial cell culture, were removed from the medium (EM and DM, supplementary information Table S1) due to their suppression of differentiation towards EC cells (supplementary information Figure S1). A8301, a TGF- β receptor signaling inhibitor, was added to support differentiation and maintain an intestinal-cell morphology in the monolayer cultures.^{10,11} The EE (ChgA⁺) and EC (5HT⁺) cells were present at a density of 22 ± 5 and 10 ± 3 cells/mm², respectively (supplementary information Figure S2A and B). Accordingly, the total number of EC cells in a 96-well insert (surface area = 11 mm²) was 110 ± 33 . To determine if this cell density was sufficient for functional assays, the monolayers were stimulated with vehicle (DMSO) or forskolin (10 μ M) apically for 4 h

followed by 5HT quantification in the basal and apical media. The release of 5HT from the apical side (0.42 ± 0.18 ng/mL) was less than that from the basal side (0.88 ± 0.28 ng/mL, supplementary information Figure S2C). This is consistent with the physiologic role of EC cells in which they secrete ligands into the bloodstream to modulate bodily functions. For this reason, we characterized 5HT secretion from the basal side of the epithelial monolayer. In three independent experiments (supplementary information Figure S2D), 5HT secretion induced by forskolin was 0.97 ± 0.34 , 0.88 ± 0.28 , and 0.60 ± 0.23 ng/mL, respectively, corresponding to 3.6 $\times$, 4.6 $\times$ and 2.4 $\times$ induction over the vehicle controls (0.27 ± 0.10 , 0.19 ± 0.06 ng/mL, and 0.25 ± 0.07 respectively). Significant inter-experiment variability was observed due to high standard deviation and low signal-to-noise in the measurements: the difference between vehicle and forskolin of the first and third experiments were not statistically significant ($p > 0.05$, t-test) while that of the second experiment was ($p < 0.05$). The high standard deviation was due to a number of possible sources: variability and sensitivity of the 5HT ELISA assay itself, well-to-well variation in EC cell number, 5HT contamination from residual fetal bovine serum in the well, and spontaneous background secretion of 5HT by the cells. To improve the reliability of the 5HT secretion assay and its signal-to-background ratio, the number of ECs in monolayers needed to be further increased.

In hopes of accomplishing this increase, culture under ALI conditions was attempted. ALI culture has been recently applied to intestinal monolayer cultures to improve the secretory cell lineage differentiation with a focus on goblet cells and mucus secretion.^{20–22} Under ALI culture, all fluid is pipetted from the reservoir above the apical cell surface. However, *in vivo*, the colon has significant fluid and other hydrated contents as well as intermixed gases. Water and electrolyte homeostasis of the colonic mucosa are carefully balanced with water moving into and out of the lumen as needed. Thus, it was likely that the water/electrolyte balance of the intestinal monolayers cultured under ALI alone was disturbed. Vasoactive intestinal peptide (VIP) plays a critical role in water balance in the intestine and when added to ALI cultured-intestinal monolayers promotes water transport from the basal to apical side with maintenance of a thin layer of fluid (~ 0.4 mm in thickness) over the apical tissue surface.²¹ To determine whether ALI culture plus VIP might improve EE lineage differentiation, the colonic monolayers were differentiated under four strategies outlined in Figure 1A, and the presence of EE and EC cells in monolayers were assessed by immunofluorescence staining (Figure 1B). Compared with submerged culture (EE: 22 ± 5 cells/mm², EC: 10 ± 3 cells/mm²), ALI alone significantly increased the number of EE (259%) and EC (260%) cells (Figure 2A,B). Addition of VIP to submerged cultures also increased the number of EE (177%) and EC (180%) cells, but to a lesser extent than ALI (Figure 2A,B). This result suggests that VIP favors cell differentiation toward secretory cell lineages, which is consistent with previous *in vivo* and *in vitro* studies on the effect of VIP on goblet cell production.^{21,25} An additive effect was observed by combining ALI and VIP, which significantly increased the number of EE (377%) and EC (430%) cells compared with the traditional submerged culture without VIP (Figure 2A,B). The fraction of EC amongst the EE cells remained in the range of 45–51% under these differentiation conditions, which is consistent with their fraction in the body where EC cells make up 50–70% of the EE population in the colon and rectum.² To determine whether EC cell enrichment also increased stimulated 5HT secretion, the monolayers were incubated with vehicle (DMSO) or

forskolin (10 μ M). The 5HT secretion was found to correlate with the observed number of EC cells (Figure 2C). 5HT secretion per cell was not significantly different under the four conditions, suggesting that VIP-ALI increased the number of EC cells in each well rather than the amount of 5HT secreted per cell (supplementary information Figure S3). The monolayer generated from VIP-assisted ALI culture significantly increased 5HT secretion under forskolin stimulation (390% higher than that generated from the submerged culture). For the VIP-assisted ALI culture, forskolin increased 5HT secretion by 6.1 $\times$ over the vehicle control, greatly improving the likelihood that the platform could be used to study EC cell stimulation and inhibition.

An additional advantage of VIP-assisted ALI culture was improved barrier integrity (Figure 2D). Among four differentiation strategies, VIP-assisted ALI culture generated monolayers possessing a physiologic barrier integrity with low permeability coefficients ($P_{app} < 4 \times 10^{-7}$ cm/s) for lucifer yellow, a small molecule used to test the permeability of monolayer cell cultures. Under these media conditions, submerged cultures (in the absence or presence of VIP) failed to generate impermeable monolayers. Some intestinal-cell culture systems demonstrate hypoxia at the cell surface and this aspect may have been a contributing factor under these culture conditions.²⁶ ALI culture alone also failed to produce low-permeability monolayers and these monolayers appeared to become desiccated. VIP-assisted ALI culture maintained a thin layer of fluid (0.4 mm thick) at the apical monolayer side potentially eliminating the stresses of ALI alone. VIP may also offer additional beneficial roles in maintaining intestinal epithelial barrier homeostasis by reducing epithelial-cell inflammation and tight junction disruptions.^{25,27} The high integrity of VIP-assisted ALI monolayers enables directional secretion of hormones as well as selected application of inhibitors and stimulants to a single side of the monolayer to better mimic physiologic exposure to drugs and food stuffs.

Effect of differentiation duration

As the generation and storage of 5HT inside the granules of EC cells increases with EC cell maturation, the level of 5HT secretion may be impacted by the time the monolayers remain in culture under differentiation conditions. To identify the optimal differentiation duration, the monolayers were subjected to VIP-assisted ALI culture for 3–7 days. Forskolin-initiated 5HT secretion increased with differentiation time as did the background basal secretion (Figure 2E). Since the cells rapidly cease division when placed into differentiation medium (without growth factors), the change in 5HT secreted was most likely due to EC cell maturation rather than increased cell number. The barrier integrity remained ($P_{app} < 4 \times 10^{-7}$ cm/s) for all samples, except for those at 7 days of differentiation at which time the cells reach the end of their life span and begin to undergo apoptosis. Five days of differentiation was optimal as this duration yielded the maximal 5HT induction (5.7 $\times$ relative to background) with excellent monolayer barrier integrity. Unless otherwise specified, 5 days of differentiation were used in subsequent experiments.

Passage and donor variation

Stem cell passage number is an important consideration when designing an assay using primary cells as they are vulnerable to replicative senescence when multiplying *in vitro*.²⁸

To determine whether the passage number affected 5HT secretion, colonic stem cells were plated onto the inserts at different passage numbers (7, 9, 11, 13 and 15). No significant variation of 5HT secretion level (both vehicle and forskolin stimulated) was observed (Figure 2G). The induction of 5HT secretion (forskolin/DMSO) was approximately 5×. As is standard practice, the stem cells were discarded after passage number 16 because of the increasing probability for chromosomal abnormalities due to *in vitro* culture.²⁹

Primary stem cells derived from different donors are known to have varying behavior in culture depending on the age, gender, and health of individuals from whom the tissue was derived.³⁰ To study donor variation, stem cells from the transverse colons of 5 donors of different age and gender (Figure 2H) were used to investigate donor variability in terms of 5HT secretion. Significant variation in 5HT secretion quantity (both vehicle and forskolin stimulated) was observed (Figure 2I). Low 5HT induction (forskolin/DMSO, <3.6) was observed for donors 3 and 4, while high 5HT induction (>5.0) was present for donors 1, 2, and 5. Significant donor variation suggests that 5HT secretion behavior is complicated and may be associated with many other factors besides gender and age, for example, donor body mass index, blood sugar level, dietary habit and health status. Therefore, a full investigation of donor variation needs to be established for these primary cell-based assays. As the cells from donor 2 demonstrated excellent 5HT secretion, these cells were used for the subsequent screening experiment.

Small scale screening of pungent food ingredients

In vivo, EC cells are characterized by a pyramidal shape with a large basolateral surface. This pyramidal shape was recreated in the *in vitro* monolayer platform (Figure 3A). Like other EE subtypes, EC cells sense luminal contents, particularly dietary compounds and metabolites, through sensory receptors on their apical surface, and then release 5HT from their basal surface into the lamina propria (Figure 3B).⁷ The EC-enriched, impermeable monolayers enable selective application of food compounds to the cells apical surface to mimic physiologic exposure. In contrast, the apical surface of the widely used intestinal organoid system is not accessible to exogenous compounds since the lumen is in the interior of the organoid with only the basal surface of the cells accessible to exogenous compounds. To demonstrate the utility of the monolayer system, 13 types of pungent plant food ingredients (Figure 3C) were tested for their ability to modulate 5HT production in response to luminal or apical stimuli (Figure 3D). Among these compounds, curcumin and forskolin were found to be most potent with 5HT induction of 7.3× and 4.9×, respectively. Cinnamaldehyde and bradykinin also significantly increased 5HT secretion, but to a lesser degree with induction of 1.8× and 1.4×, respectively. Curcumin stimulated the 5HT secretion in a dose-dependent manner with a median effective dose (E_{50}) of 51 μ M (Figure 3E). Other tested compounds did not significantly stimulate the secretion of 5HT.

Presence of L cells within intestinal monolayers formed under VIP-assisted ALI culture

Besides EC cells, L-cells are major regulators of the gut-brain interactions.³¹ L-cells co-secrete peptide hormones glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) in response to the ingestion of food. GLP-1 stimulates the secretion of insulin from pancreatic β -cells, decreases gastric emptying and increases satiety. PYY slows GI motility and reduces

food intake. L-cells were also identified in the monolayers at a density of $12 \pm 2 \text{ mm}^{-2}$ and constituted ~14% of the EE population (supplementary information Figure S3). Therefore, this EE-enriched platform has the potential for medium- and high-throughput screening of dietary compounds and metabolites that alter the secretion of not only 5HT, but other intestinal hormones such as GLP-1 and PYY.

CONCLUSIONS

Normal adult IESCs from human transverse colon were differentiated to create functional monolayers enriched with EE and EC cells on a porous membrane. A simple strategy, VIP-assisted ALI culture, significantly enhanced the number of EC cells and 5HT secretion by 430% and 390%, respectively, over the traditional submerged culture. Additionally, VIP-assisted ALI culture produced high barrier integrity monolayers enabling the strict segregation between the apical and basal aspects of the epithelium so that cells could be stimulated and assayed from either side independently. Basal 5HT secretion was highly inducible to stimulation by forskolin and curcumin applied apically of approximately 5× and 7×, respectively. Cells from a single donor displayed reproducible 5HT secretion over different passage numbers. However, significant variation was found among monolayers derived from 5 different donors. The ALI-VIP monolayers were readily formed on a 96-well platform to support a small-scale screening of 13 pungent food ingredients. This EE and EC-enriched model made up of primary human cells is anticipated to be of high value for high throughput screening of stimuli present in the lumen that alter the secretion of intestinal hormones and hence impact human eating behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Number of EE and EC cells on human stem-cell-derived intestinal epithelial monolayers is dependent on the culture conditions and differentiation strategy. (A) Schematic showing the process to generate fully differentiated, confluent monolayers derived from primary intestinal epithelial stem cells by four differentiation strategies: submerged (Sub) and air-liquid-interface (ALI) in the absence or presence of VIP. (B) Representative low-magnification fluorescence microscopic images of monolayers. Inserts show high-magnification images. Colors as follows: chromogranin A (ChgA, EE marker), red; 5HT (EC marker), green; Hoechst 33342-stained DNA, blue.

Figure 2.

Characterization of the EC-enriched monolayers. (A) Quantification of the number of EE (ChgA⁺) cells in monolayers generated by four differentiation strategies. (B) Quantification of the number of EC (5HT⁺) cells. (C) 5HT secretion from monolayers after apical exposure to DMSO vehicle and 10 μ M forskolin for 4 h. (D) Box plot of apparent permeability coefficient (P_{app}) of monolayers for lucifer yellow. The dashed line marks a P_{app} of 4×10^{-7} cm/s, a commonly accepted threshold for barrier integrity. (E-F) Effect of differentiation duration (3–7 days) on 5HT secretion (E) and barrier integrity (F, box plot). (G) 5HT secretion from monolayers generated from stem cells at different passage number. (H) List of information (age and sex) of stem cells from five donors. (I) 5HT secretion from monolayers generated from transverse colonic stem cells from five donors. In (D) and (F),

sample number = 6 for each condition. In other figures, sample number = 3 for each condition. * $p < 0.05$, ** $p < 0.005$.

Figure 3.

A small-scale compound screen for modulation of 5HT secretion. (A) Confocal fluorescence microscopy (XY and XZ) showing triangular or pyramidal EC cell morphology. EC cells exhibit yellow color due to co-staining with 5HT (green) and ChgA (red). DNA is stained with Hoechst 33342 (blue). (B) Schematic of EC cell showing that dietary compound can stimulate the apically located receptors followed by the release of 5HT from the basal surface. (C) List of 13 dietary pungent ingredients, plant source and working concentration. (D) 5HT secretion from monolayers after apical stimulation with dietary compounds for 4 h. Sample number = 3 for each condition. * $p < 0.05$, ** $p < 0.005$.