REVIEW ARTICLE

ENTEROENDOCRINE CELLS- UNFOLDING THE MYSTERY

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ARTICLE INFO	ABSTRACT
	The enteroendocrine cells form the largest system of endocrine cells in the body scattered diffusely in the gastrointestinal mucosa from stomach to rectum. These cells and their secretary products are essential for wide array of functions including digestion, nutrient absorption, immune response to luminal chemicals, toxins and microbes, luminal sensing via gut-brain axis, gut motility and epithelial renewal. Recently several molecular mechanisms underlying these diverse functions as well as underlying the diseases like irritable bowel syndrome, obesity and type 2 diabetes with possible role of enteroendocrine cells have been elucidated providing novel therapeutic targets. In this review we elaborate current knowledge on development, morphology, secretions, functions, molecular mechanisms and clinical implications of enteroendocrine cells in the gastrointestinal tract of the adult human being.

INTRODUCTION

Enteroendocrine cells (EECs) are the hormone secreting cells situated diffusely in the wall of gastrointestinal tract comprising approximately 1% of epithelial cells in the gut (Rehfeld, 2004, Sternini *et al*., 2008). The enteroendocrine system of the gut can be considered in three broad parts: the gastric, proximal intestinal and distal intestinal, colon. There is a specific distribution pattern of EEC types in each part (Dockray, 2003). Many of these cells possess cytoplasmic processes for contact with adjacent cells in a neurocrine manner (Hauso *et al*., 2007). The enteroendocrine cells form the largest system of endocrine cells in the body, presently comprising 16 cell types most of which have been named by an alphabet (Helander and Fandriks, 2012). Peptides/amines secreted by EECs play an important role in the regulation of food intake, energy expenditure, carbohydtare and lipid metabolism (Skipper and Lewis, 2000; Ramos *et al*., 2012).

These hormones are altered in diseases like obesity and type 2 diabetes, and thus can be possible targets for treatment of these diseases. The molecular mechanisms in response to luminal contents have become apparent over the last few years. Hence dietary modifications are expected to modulate gut hormone responses preventing or treating metabolic diseases. The molecular alterations in the gut pathologies such as irritable bowel syndrome and carcinoids are being clearly elucidated indicating the possible role of EECs and thus new avenues of treatment for these diseases (Ramos *et al*., 2012). In view of such rising importance of EECs in health and disease, their development, morphology, functions and clinical implications are revealed in following sections.

Historical Glimpse

Mucosa of the stomach, small and large intestine contains scattered cells that have endocrine function. Heidenhain (1870) was first to observe these cells in the stomachs of rabbit and dog. These were earlier known as basal granulated cells and were named either after their founders (Heidenhain cells,

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Nussbaum cells, Kultschitsky cells, Schmidt cells) or according to their staining characteristics (enterochromaffin, argentaffin, argyrophile cells). Two types of staining methods were used for these cells - Argentaffin methods (e.g. Gomori-hexamine-silver, Masson-Hamperl) and Argyrophile methods (e.g. Singh, 1964). On the basis of these reactions the EECs were grouped into two types- argentaffin or enterochromaffin cells (cells that are both argyrophile and argentaffin) and enterochromaffin like cells (cells that are only argyrophile). The first secretion product, the Serotonin, of the enterochromaffin cell was documented by Erspamer and Asero (1952). Since then a number of other hormones secreted by EECs and thus several types of EECs based on their secretions were recognized. A nomenclature for EECs was adopted at Wiesbaden in 1969, based on their electron microscopic appearance (Forssmann *et al.,*, 1969; Creutzfeldt, 1970). Seven basic types such as A cells, D cells, EC and ECL cells, G cells, L cells and S cells were recognized, functions of some of which were still uncertain. Bencosme *et al.* (1973) demonstrated that with modified Herlant's tetrachrome stain, different endocrine cells in the upper GI mucosa can be concurrently recognized (Masson's trichrome is also suitable for the same). EC and ECL cells stain brown-yellow, G cells stain pink and D cells take pale blue stain in human stomach with this stain. D'Adda and Bordi (1988) demonstrated that all the EECs can be stained by immunohistochemical technique using markers such as chromogranin A, neuron specific enolase (NSE) and synaptophysin. Later by use of specific IHC stains and ultrastructural properties, several cell types and subtypes have been characterized in the GI tract (Furness *et al.*, 2013).

Development and Differentiation

Previously EECs were thought to develop from the neural crest hence older term neuroendocrine cells was used (Gunawardene *et al.*, 2011). Andrew (1998) experimentally proved that these cells are not derived from the neural crest but are developed from local endoderm. EECs develop from the same pluripotent stem cells as the other three cell lineages of the intestinal epithelium: absorptive enterocytes, goblet cells and paneth cells (Gordon, 1993). Gestational time of appearance EECs in human fetal stomach was described by various authors (Stein *et al.*, 1983; Oberg, 1998).

EECs first appear in the gastric epithelium (at 9 to 10 weeks) as small proto-endocrine cells. Fairly differentiated EC-, D- and GLI-cells are observed by the 10th week. Pyloric G-cells and oxyntic ECL-cells appear later around the 14th week. Although the molecular mechanisms that regulate the differentiation of EECs have not yet been completely elucidated, studies have shown that Notch signaling controls EECs differentiation. It mediates lateral inhibition in the developing gut epithelium. Notch signaling mechanism for controlling EECs production is probably critical both during embryonic development and constant gut epithelium renewal in the adult. Key transcription factors such as Pax4, Pax6, BETA2/NeuorD, Pdx1, Gfi, Nkx2.2 and Sox9 have been identified in EEC differentiation. Differentiation of EECs is controlled by the sequential expression of three basic helix-loop-helix motifs, Math1, Neurogenin 3 (Neurog3) and NeuroD. These findings may therefore have important implications for therapeutic strategy (Skipper and Lewis, 2000; Gunawardene *et al.*, 2011).

EEC- Morphology, Distribution, Ultrastructure, Secretions and Functions

Enteroendocrine cells are scattered diffusely in the epithelium of the gastrointestinal mucosa from the cardia to the rectum (Rindi *et al.*, 2004). The concentration of EECs is highest proximally and falls steadily from stomach to colon rising again within the rectum. Common features of EECs include apical cytoplasmic process with microvilli. EECs of the large intestine, in addition, may show the basal processes (Gunawardene *et al.*, 2011). EECs are characterized by the presence of secretary vesicles: large dense core vesicles (LDCV) and synaptic-like microvesicles (SLMV), components of which can be used as general markers for EECs using immunohistochemistry (IHC) and include chromogranin A (LDCV marker) and synaptophysin (SLMV marker). Other general markers for EECs include cytosolic markers NSE and protein gene product 9.5 (Rindi *et al.*, 2004). EEC subtypes were previously distinguished by differences in their secretory vesicles on electron microscopy.

Table 1. Enteroendocrine cells of the gastrointestinal tract

EEC Type	Situation	Secretory Product	Function
A (X-like) cells and subtypes	Stomach	Ghrelin, nesfatin-1	Appetite control, growth hormone release
Enterochromaffin-like cells	Stomach	Histamine	Stimulation of gastric acid secretion
G cells	Stomach	Gastrin	Stimulation of gastric acid secretion
D cells	Stomach, small intestine (and pancreas)	Somatostatin	Inhibition of gastrin release (stomach); modulation of insulin release (pancreas)
Enterochromaffin cells	Stomach, small and large intestine (Most Common EEC)	5-HT	Facilitation of intestinal motility reflexes and secretion; triggering of emesis and nausea in response to toxins, visceral sensation
I cells	Proximal small intestine	CCK, (5-HT)	Activation of gallbladder contraction and stimulation of pancreatic enzyme secretion
K cells, and subtypes	Proximal small intestine	GIP	Stimulation of insulin release
L cells, and subtypes	Distal small intestine, colon	GLP-1, GLP-2, PYY, glycentin, oxyntomodulin, (5-HT)	Stimulation of carbohydrate uptake, slowing of intestinal transit, stimulation of mucosal enterocyte proliferation, appetite regulation, insulin release
M cells	Small intestine	Motilin	Initiation of migrating myoelectric complex
N cells	Small and large intestine	Neurotensin	Inhibition of intestinal contractions
P cells	Stomach	Leptin	Appetite regulation, reduction of food intake
S cells	Proximal small intestine	Secretin	Reduction of acidity in upper small intestine by stimulation of bicarbonate release

EEC- Enteroendocrine cell; 5-HT- Serotonin; CCK- Cholecystokynin; GIP- Glucose-dependent Insulinotropic Peptide; GLP- Glucagon Like Peptide;
PYY- Peptide YY (*Modified from Gunawardene et al., 2011; Furness et al., 2013*)

The peptide/amine content of a secretory vesicle is the most specific feature of EEC subtype and has formed the basis for their classification (Solcia *et al.*, 1978). Immunohistochemical and ultrastructural techniques have demonstrated different cell types dispersed in the GI tract. Characteristics of these cell types are summarized in Table 1. Several cell types express a specific hormone but there are a few cell types which coexpress two or more hormones. A few EEC types, such as A, K and L cells, have subgroups that contain different combinations of products (Furness *et al.*, 2013). EECs are involved in a variety of processes such as digestion, absorption, secretion of the glands, GI motility, and affect the blood flow through the blood vessels and the gut epithelial cell cycle. Foetal endocrine cells function as stem cells capable of differentiating into one or more types of endocrine cells. They are important for the development and differentiation of the digestive system and pancreas by exerting trophic effects of some peptide hormones. Targets for action of EECs include secretory cells, smooth muscle, proliferating cells and signal to the brain as gut-brain axis with varied functions (Rindi *et al.*, 2004).

A (X-like) cells and subtypes

'A' cells constitute approximately 20 % of all endocrine cells in the human gastric glands (Simonsson *et al.*, 1988; Cui and Waldum, 2007)). These cells produce ghrelin, a novel growth-hormone-releasing peptide, hence also known as ghrelin cells or Gr cells (Date *et al.*, 2000). Ghrelin increases growth hormone secretion, food intake, gastric motility, gastric acid seretion, weight gain and positive cardiovascular effects. Rindi *et al.* (2002) stated that ghrelin cells are demarcated in developing gut from 10th gestational week and are characterized by round, compact, electrondense secretory granules of P/D1 type in man, A-like type in the rat and X type in the dog. Zhao and Sakai (2008) identified two types of ghrelin cells as opened- and closed-type. These cells also produce nesfatin-1, a protein fragment that inhibits food intake (Stengel *et al.*, 2009).

Enterochromaffin-like (ECL) cells

The ECL cells are the predominant endocrine cell type exclusive to oxyntic mucosa constituting 1–3% of the fundic epithelial cell volume. Shintani and Watanabe (2007) observed that ECL cells were found not only in the fundic region, but also in the intermediate and pyloric regions of the stomach. These cells do not contact the lumen (closed type). ECL cells can be detected by Grimelius staining. The presence of histamine was detected in gastric ECL cells in 1986 by Hakanson *et al*. IHC studies later confirmed the location of these cells in the lower third of the gastric glands (Simonsson *et al.*, 1988). ECL cells produce histamine, chromogranin A/pancreastatin, synaptophysin, histidine decarboxylase and calbindin (Gastrocalcin). Gastrin stimulates ECL cells to release histamine which in turn stimulate the parietal cells to produce gastric acid. The most important inhibitor of the ECL cell is somatostatin from oxyntic D cells.

G cells

'G' cells are exclusive to the antropyloric mucosa of the stomach situated in close proximity with D cells and secrete gastrin (Bordi *et al.*, 2000). The ultrastructural features depend on functional activity of G cells. The round secretory granules measure 200-250 nm in diameter with variable electron density (Mitschke, 1977). Gastrin stimulates gastric acid secretion by parietal cells of oxyntic mucosa. The secretion and expression of gastrin are under the paracrine control of somatostatin, produced by D cells situated in close contact with G cells. D cells also contain neuronal nitric oxide synthase and regulate G cells apoptosis (Larsson, 1999).

D cells

'D' cells are diffusely present throughout the GI tract (Cui and Waldum, 2007) with highest frequency in the duodenum and lower levels in the ileum and colon. D cells are concentrated in the lower third of the crypts and secret somatostatin (Gunawardene *et al.*, 2011). Hauso *et al.* (2007) described the ultrastructure of D cells in the large intestine with one apical and one basal extension. This differs from the appearance of D cells in the stomach which have 2 or 3 cytoplasmic extensions. The secretory vesicles of D cells are 200–400 nm in diameter. Somatostatin is the inhibitory hormone that reduces the secretion of all other gut hormones and also exocrine functions of the GI tract and pancreas.

Enterochromaffin (EC) cells

Enterochromaffin (EC) cells are the most abundant EECs of the gut and are distributed widely from stomach to rectum. Originally named because of their affinity for chromium salts, they secret serotonin (5-HT). Voutilainen *et al.* (2002) observed that EC cells are the only endocrine cell type present at the gastric cardia mucosa and proposed that they may regulate the lower oesophageal sphincter physiology. EC cells are approximately 8 micrometres in size and pyramidal in shape with an apical process. LDCVs are present in both apical and basal parts of the cell (Gunawardene *et al.*, 2011). Dual staining immunofluorescence technique for acridine orange (AO) and TPH (tryptophan 5-hydroxylase, the initial enzyme in 5-HT biosynthesis) has recently emerged as specific marker for EC cells (Gunawardene *et al.*, 2011). Most of the 5-HT content of body is within the GI tract (highest in the rectum), the majority of which is contained within the EC cells. EC cell secretion is partly neurally regulated and is also triggered by luminal fatty acids and toxins (Ferrara *et al.*, 1987). 5-HT released by EC cells produce peristalsis, triggers emesis and nausea in response to toxins. It causes increased secretion and motility, and reduction in appetite.

I cells

'I' cells are located in the proximal small intestine, predominantly in the duodenum, and release Cholecystokinin (CCK), a peptide hormone. I cells are characterized by small (166 +/- 38 nm) secretory granules with dense core separated from its enveloping membrane by a thin clear space (Usellini *et al.*, 1985). CCK stimulates gallbladder contraction, exocrine pancreatic secretion, delayed gastric emptying and satiety.

K cells and subtypes

'K' cells are scattered in the proximal small intestine and secret Glucose-dependent insulinotropic polypeptide (GIP), a

peptide that stimulates insulin secretion from pancreas in response to luminal carbohydrates and lipids (incretin effect). GIP also regulates appetite and gut motility. The highest level of GIP mRNA was detected in the duodenum and jejunum (Tseng *et al*., 1993). K cell shows round, homogeneous, osmiophilic granules with closely applied membrane and a mean size of 188 nm (Usellini *et al*., 1984a).

L cells and subtypes

'L' cells are diffusely present from duodenum to the rectum, concentrated in the distal ileum and colon. They constitute the second largest population of EECs in the colon and contribute to 14% of the EEC population in the rectum. These cells are bottle or flask shaped, are of open type and exhibit microvilli as well as basal processes. L cells secret proglucagon-derived peptides (PGDPs) i.e. glicentin, GLP-1, GLP-2 and oxyntomodulin and peptide YY. GIP and GLP-1 colocalize in the mid-small intestinal cells which are called K/ L cells (Gunawardene *et al*., 2011). PYY reduces intestinal and colonic transit, reduces gastric emptying, gastric acid and pancreatic exocrine secretion. GLP-1 exerts incretin effect by which it stimulates pancreatic insulin release in response to ingested glucose. GLP-1 also has an inhibitory effect on gastric acid and pancreatic exocrine secretion. GLP-2 and Glicentin stimulates mucosal epithelial proliferation.

M cells

The motilin (M) cells are scattered in the epithelium of upper small intestine and are characterized by relatively small (180 nm) solid round granules with homogeneous core and closely applied membrane with prominent perinuclear microfilaments (Usellini *et al*., 1984b). These cells secrete motilin which increases GI motility.

N cells

The neurotensin secreting N cells are diffusely present in the epithelium of the jejunum-ileum and colon. This third most frequent endocrine cell in small intestine contains several basal electron dense cytoplasmic granules. These neurotensin granules are round, highly electron dense with mean diameter of 260--290 nm (Sundler *et al*., 1977). Neurotensin inhibits intestinal contractions.

P cells

P cells are exclusive to the stomach and are found in both the antral and oxyntic mucosa (Cui and Waldum, 2007). Cinti *et al*. (2000) and Buyse *et al*. (2004) demonstrated that the secretory granules of endocrine P cells and chief cells of the human gastric mucosa contain leptin which plays a key role in the regulation of food intake, energy expenditure and sympathetic activity hence can be the target for treatment of obesity. Leptin is also involved in the regulation of angiogenesis, hematopoiesis, reproduction, bone formation, and immune response (Ahima and Osei, 2004).

S cells

Secretin (S) cells are scattered in the mucosa of duodenum and jejunum. These contain large (mean diameter 299 nm)

irregular granules. Majority of the granules show secretin and chromogranin A in a homogeneous core. Other granules show a targetoid pattern due to an inner, argyrophobe body surrounded by an argyrophil mantle and represent a distinctive ultrastructural marker of the secretin cell. About 1/3 of secretin cells also show serotonin immunostaining (Usellini *et al*., 1990). Secretin reduces acidity in upper small intestine by stimulation of bicarbonate release

Molecular Mechanisms and Clinical Implications

Understanding of the EECs and their secetory products has laid the foundation for digestive physiology. Alterations in the structure and function of these cells are associated with GI pathologies. Nutrient-sensing and chemosensing receptors on enteroendocrine cells have been identified which sense nutrients and harmful substances in the lumen and prepare the gut to absorb them or initiate a protective response. They might also participate in the control of food intake through the activation of gut-brain neural pathways by releasing satiation peptides (Engelstoft *et al*., 2008; Sternini *et al*., 2008; Cummings and Joost, 2007). Leptin plays a key role in the regulation of food intake, energy expenditure and sympathetic activity. 5-HT can cause reduction in appetite. Hence the leptin and 5-HT receptor agonists may be effective in the treatment of obesity (Garfield and Heisler (2009). Glucose given orally induces secretion of GLP 1 and GIP (the incretin hormones), which in turn regulate appetite, insulin secretion, and gut motility is a good example of gastrointestinal chemosensation (the "incretin effect"). Modulating the secretion of these peptides from gut EEC may provide new avenues for treating obesity, diabetes and malabsorption syndromes (Gunawardene *et al*, 2011; Kokrashvili *et al*., 2009).

Voutilainen *et al*. (2002) observed that EC cells are the only endocrine cell type present at the gastric cardia mucosa and they may regulate the lower oesophageal sphincter physiology. We propose further studies to know the role of these cells in gastro-oesophageal reflux disease.

5-HT is important in response of EEC to chemical, mechanical and other stimuli in the gut lumen. It increases secretions and peristalsis and signals to brain via vagal afferents (via 5-HT3 receptors) causing nausea. Hence Ondansetron, 5-HT3 receptor antagonist, has anti-emetic effect. EECs play an important role in mucosal immunity and repair. Inflammation in the gut is associated with an alteration in EECs and their secretions. Decrease in number of D cells and circulating somatostatin levels has been observed in patients with chronic gastritis and IBD. This indicates a role of the immune system in EEC regulation (Gunawardene *et al*., 2011). The studies demonstrate that 5-HT activates the immune cells to produce proinflammatory mediators (Khan and Ghia, 2010). Reduced levels of peptide YY in IBD patients have been observed by El-Salhy *et al*. (2002), which may contribute to diarrhoea in these patients owing to increased gastric emptying, intestinal motility and intestinal secretions. Glicentin on the other hand acts as a protective mucosal agent, preventing bacterial translocation of enteric bacteria when added to cell lines in vitro (Chiba *et al*., 2007). These findings lead to understanding and ultimately to improved therapeutic strategies in inflammatory gut disorders including Irritable Bowel

Syndrome, infectious enteritis, and inflammatory bowel disease.

Tumors of the EECs are referred as "carcinoids". Oberndorfer used this term in 1907 for epithelial tumors in the gut that have a relatively monotonous structure and are less aggressive than carcinomas (Alexiev *et al.*, 2007). ECL cells are highly sensitive to the trophic influence of gastrin and undergo hyperplasia in hypergastrinemic states. Chromogranin A and its products may act as growth promoting agents in ECL-cell hyperplasia or gastric carcinoids (D'Adda *et al.*, 1988). Studies suggest that a few enteroendocrine cells migrate down to or stay at the crypt base and express stem cell and postmitotic endocrine markers. Further investigation of the function of this group may provide understanding of development of EECs and enteroendocrine tumorigenesis (Sei *et al.*, 2011). New evidence suggests a possible relationship between EECs of the lower GI with the development and progression of primary colorectal adenocarcinoma. Further research should focus on this association leading to new preventive and diagnostic targets (Gunawardene *et al.*, 2011).

Studies have shown that Notch signaling controls EECs differentiation by lateral inhibition in the developing gut epithelium. This mechanism may be important both during embryogenesis and gut epithelium renewal in the adult. These findings may therefore have important implications in epithelial repair and tumorigenesis (Skipper and Lewis, 2000). EECs still remain mysterious group of cells. An understanding of their ontogenesis, relationships, metabolism, though being extensively studied, is yet incomplete. Future research should illustrate their importance, interactions and therapeutic potential.

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