Review

The small intestinal mucosa and its stem cells

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Proceedings

Abstract

In the first part of this review a brief summary of the embryology and histology of the gastrointestinal tract is provided. In the second part intestinal stem cells (ISCs) are discussed. Several signaling pathways play a crucial role in the crypt base in the regulation of ISC proliferation and self-renewal; Wnt, Notch, BMP, Ephrin, JAK/STAT1, PTEN, AKT, PI3K and many more. Numerous investigators are involved in studying the location, number, and behavior of ISCs within the base of the intestinal crypts. Several markers are espressed by ISCs. Among these, Leucine-rich-repeat-containing G-protein-coupled receptor-5 (Lgr5), Sox9, Prominin-1, DCAMKL-1, EphB2, p-PTEN, p-AKT, Fgfr3, m-TER, and CD44. Stem cell therapy has shown promise for the treatment of some diseases characterized by tissue damage with ischemic and inflammatory lesions like inflammatory bowel disease (IBD) and necrotizing enterocolitis (NEC).

Keywords

Small intestinal mucosa, embryogenesis, crypt-villus axis, stem cells, signaling pathways, regenerative medicine.

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Embriology and histology

The gastrointestinal tract is composed by several segments that are, from the end of the pharynx to the anus, the esophagus, stomach, small intestine, and large intestine. The general architecture of the wall of these organs is established in embryonic life, but there are some histologic changes during the development of the layers of the wall during fetal life.

The gastrointestinal tract is composed of an endoderm-derived epithelial lining, surrounded by concentric splanchnic mesoderm-derived layers of mesenchyme: the mucosa with its muscularis mucosae, submucosa, muscularis propria, subserosa or adventitia and, eventually, serosa. The development of the gastrointestinal tract is controlled by rostral-to-caudal, right-to-left, and inside-to-outside (radial) molecular genetic morphogenetic gradients, that are completed by the 10th post-fertilization week. Each segment shows a mucosa that is histologically unique to that segment and specific for the function of that segment; the other layers vary very little from segment to segment.

The mucosa is composed by the epithelium, the lamina propria and the muscularis mucosae. The esophageal mucosa is lined by non-keratinizing stratified squamous epithelium; in the stomach the mucosa has a simple columnar mucous-producing surface epithelium with gastric pits, giving rise to simple mucous cardiac and pyloric glands, as well as serous gastric glands or crypts. The small intestine has a villous mucosa with serous crypts in the lamina propria underneath the villi. The large intestine has a flat mucosa lined by a simple columnar mucous-producing surface epithelium with mucous crypts. Several neuroendocrine cells are scattered throughout the epithelium primarily in the base of the crypts [1]; at least 16 subtypes have been identified and each produces a different gastrointestinal peptide hormone [2]. The muscularis mucosae is composed of smooth muscle: this is true even in the upper esophagus where the muscularis propria is skeletal muscle.

The differentiation of the mucosa occurs in an orderly sequence similar in all segments. Before the 6th post fertilization week, the simple endodermal epithelium, that is present since the formation of the bilaminar disc, develops into a stratified columnar primordial epithelium throughout the gastrointestinal tract. During the 6th week this epithelium begins a proliferative phase, also called "vacuolization phase", and becomes multilayered and thick. Finally, during the 8th week the epithelium changes again and becomes a tall simple or pseudostratified columnar epithelium, which by 8 to 10 weeks differentiates into the epithelium specific for each segment (**Fig. 1** and **Fig. 2**).

The unique histology of the small intestine is established before the 14th week post fertilization [3]. The proliferative epithelium transiently occludes the duodenal lumen during the 7th week; then this epithelium regresses, the duodenal lumen is reestablished, and villi appear in the duodenum in the 8th week. Crypts of Lieberkühn appear in the duodenum during the 9th week. The villi and crypts spread caudally, so they appear in the terminal ileum in the 12th to 14th weeks. Brunner's glands, that appear in the first part of the duodenum, as early as the 14th week, continue to differentiate caudally throughout the rest of the duodenum during the 14th to 16th weeks.
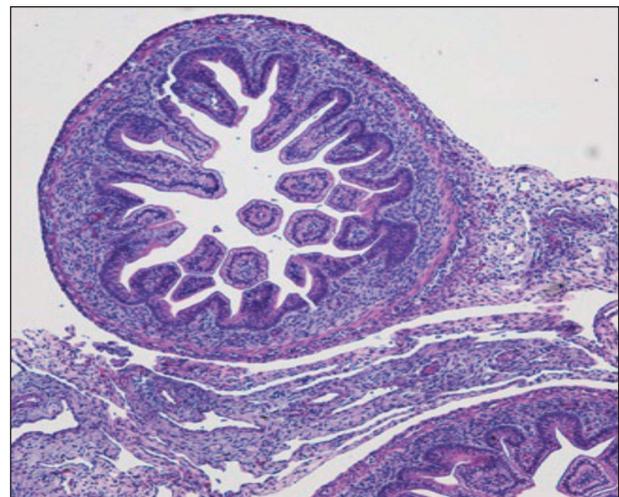
Figure 1. Small intestine at the 11th gestational week. We can observe the villi, intervillous primordial epithelium, primitive crypts of Lieberkühn, the two layers of muscularis propria, and the ring of Auerbach's plexus (H&E, 4X).
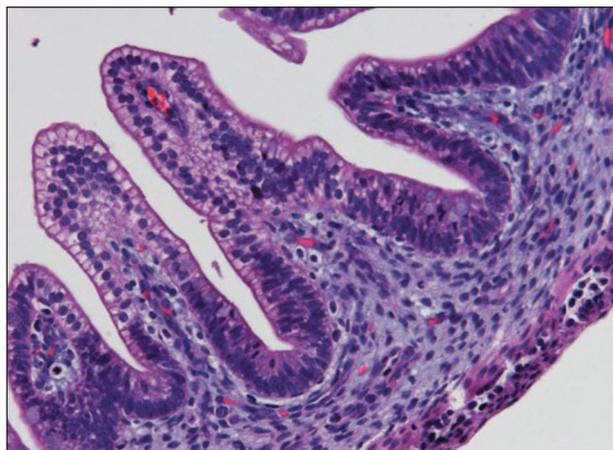
Figure 2. Small intestine at 11th gestational week. Higher magnification of **Fig. 1**. The muscularis mucosae has not yet differentiated (H&E, 20X).

During gestation, the architecture of the bowel wall is established through epithelial-mesenchymal interactions. In particular, the intestinal epithelial cells from the villus/crypt structure, its adjacent pericryptal fibroblasts that secrete various growth factors and cytokines, and mesenchyme constitute an anatomical microenvironment that, due to molecular messages from mesoderm-derived mesenchyme, the endoderm-derived epithelium evaginates to form villi, while the intervillus regions invaginate into the mucosa to form epithelial pockets: the crypts of Lieberkühn (**Fig. 3**); so, the general architecture of small intestinal mucosa is characterized by continuous villi and crypts that, in the adult, are lined by mature epithelial cells that form the functional epithelial compartment of differentiated cells no longer capable of dividing and can be categorized on the basis of their function: a) enterocytes, the dominant lineage (90% of total cells), with sharp luminal brush border that absorb nutrients, b) goblet cells that comprise 8% to 10% and secrete a protective mucus barrier, c) enteroendocrine cells, that comprise about 1% of the epithelium and produce gastrointestinal hormones and d) Paneth cells that secrete antibacterial substances.

Intestinal stem cells

The deeper part of the crypt harbors resident undifferentiated and rapidly cycling intestinal stem cells (ISCs) that form the proliferative compartment and represent a protective tissue microenvironment for the epithelial stem cells known as "niche" [4-6]. A stem cell niche can be defined as "a specific location in a tissue where
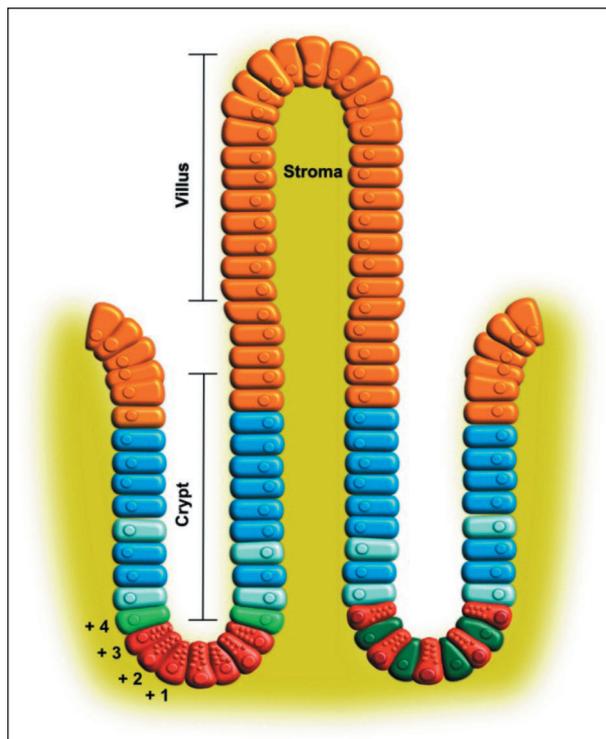
Figure 3. The intestinal cryptvillus unit. Intestinal stem cells (ISC) and Paneth cells reside at the base of the crypt. Upon these cells lie the transit-amplifying (TA) cells (yellow). The villus is lined by mature cells: enterocytes, goblet cells and neuroendocrine cells.

stem cells can reside for an indefinite period of time and produce progeny cells while self-renewing" [7]. The ISC niche is maintained by the epithelial-mesenchymal crosstalk, that occurs in spite of the basement membrane, between the proliferating and differentiating epithelial cells, the surrounding mesenchymal cells (i.e. endothelial cells, lymphoid cells, and fibroblasts/myofibroblasts) and the extracellular matrix of the lamina propria. An ISC may be defined by the ability to self-renew itself throughout long periods of time, the ability to self-mainten itself as a non-specialized cell, and the property of multipotency, i.e. the ability to generate all differentiated cell types (enterocytes, goblet cells, enteroendocrine cells, and Paneth cells) [8-10]. A proper balance of stem cell activity is needed for the normal homeostatic production of functional mature cells. In contrast, excessive stem cell proliferation can result in cancer.

Except for the Paneth cells, that reside in the bases of the crypts and are easily recognized because of their brightly eosinophilic and granular cytoplasm, the other cells migrate toward the intestinal lumen and begin to differentiate; when

the tip of the intestinal villi is reached they may undergo apoptosis and shed into the gut lumen [6].

Self-renewal and repair of adult intestinal mucosal epithelium after injury is fueled by the ISCs by producing daughter progenitor or transit amplifying (TA) cells that can subsequently differentiate into the mature cell lineages [10, 11]. As a rule, in physiologic conditions, newly produced TA cells give rise up to six rounds of cell division within 2-3 days and then they rapidly differentiate into each of the four above-mentioned mature cell types. Stem cells concomitantly self-renew and generate via asymmetric cell division, giving rise to one daughter stem cell and one TA cell, which differentiate toward mature epithelial cells [12] (**Fig. 4**).

It is likely that adult stem cells-driven tissue renewal follow three modalities of cell division (**Fig. 5**): a) symmetric division, by which two stem cells are generated; b) symmetric division, by which two TA cells are generated; c) asymmetric division, by which one stem cell and one TA cell are generated. If the gut mucosa has been damaged, or undergone irradiation or after chemotherapy, ISCs
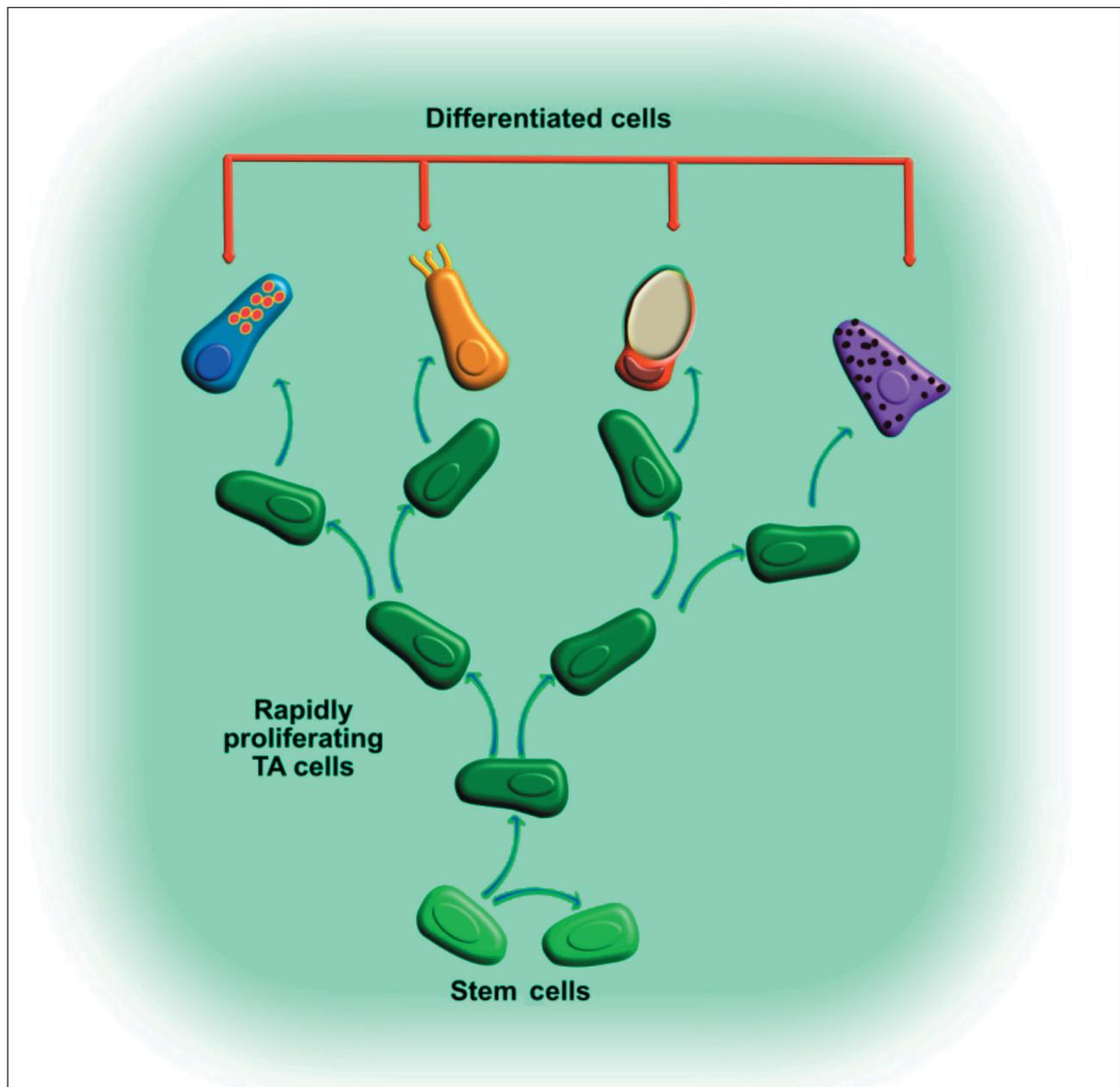
Figure 4. When stem cells divide, they undergo an asymmetric cell division into a new stem cell plus a committed daughter cell. The rapidly cycling daughter cells, also called transit amplifying (TA) cells, then undergo a limited number (some rounds) of cell divisions before differentiating into the mature, functional cell types of the adult tissue.
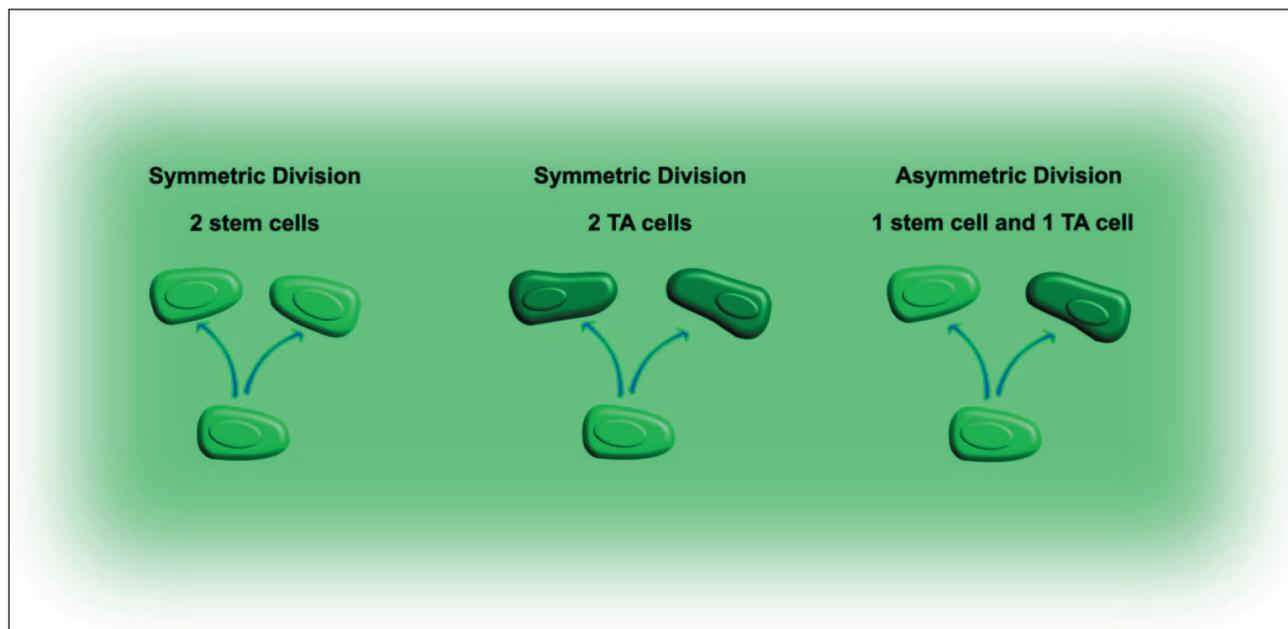
Figure 5. Adult stem cells-driven tissue renewal can follow three modalities of cell division: a) symmetric division, by which two stem cells are generated; b) symmetric division, by which two transit amplifying (TA) cells are generated; c) asymmetric division, by which one stem cell and one TA cell are generated.

likely undergo symmetrical division in order to give rise to two stem cells to replace injuried ISCs [12].

Since the early 70's, two opposing models of ISC niche have been formulated to explain the activity of stem cells and proliferative compartment: the "+4 position" model and the "stem cell zone" model [10, 11, 13].

In the "+4 position" model (**Fig. 6**) by Potten and colleagues [13], differentiated Paneth cells occupy the crypt base, and the stem cells (approximately four to six) are located just above the Paneth cells at the +4 position. The enterocytes, goblet cells, and enteroendocrine cells form the +4 cell progeny that differentiate during their upward migration onto the villi. In contrast, the Paneth cells differentiate during their downward migration from the +4 position toward the crypt base. Based on DNA-labeling reagents like BrdU, some studies suggest that label-retaining cells are located specifically at the +4 position relative to the crypt bottom, with the first three positions occupied by the terminally differentiated Paneth cells and that +4 cells are extremely sensitive to radiation, a property that functionally protects the stem cell compartment from genetic damage.

In the "stem cell zone" model (**Fig. 7**) by Cheng and Leblond [10, 11], small, undifferentiated, cycling cells (known as crypt base columnar cells, CBC cells) are wedged among the Paneth cells and seem to be the true ISCs.

A magnification of a small intestinal crypt with two different stem cell models is presented in **Fig. 8**; different markers are reported for different cells. Gradients of integrins segnaling, BMP signaling, and Wnt signaling are also reported.

Signaling pathways and intestinal stem cells

Recent studies have had an important role in understanding the molecular mechanisms that regulate the development of intestine during the embryo-fetal life as well as the homeostatic epithelial regeneration in the adult. In particular, these studies have shed light on some signaling pathways that play pivotal roles in regulating ISCs self-renewal and differentiation in normal tissue and repair of adult intestinal mucosal epithelium after injury.

Several signaling pathways play a crucial role in the crypt base in the regulation of ISC proliferation and self-renewal; Wnt, Notch, BMP, Ephrin, JAK/STAT1, PTEN, AKT, PI3K and many more.

The Wnt signaling is the first pathway that is shown to regulate ISC functions [14, 15]. The continuous stimulation of Wnt signaling pathway drives the TA cell proliferation and is correlated to the transcriptional activation of nuclear β-catenin/T cell factor (TCF) [16, 17]. Other than in mitogenic activity, Wnt signaling pathway is also involved
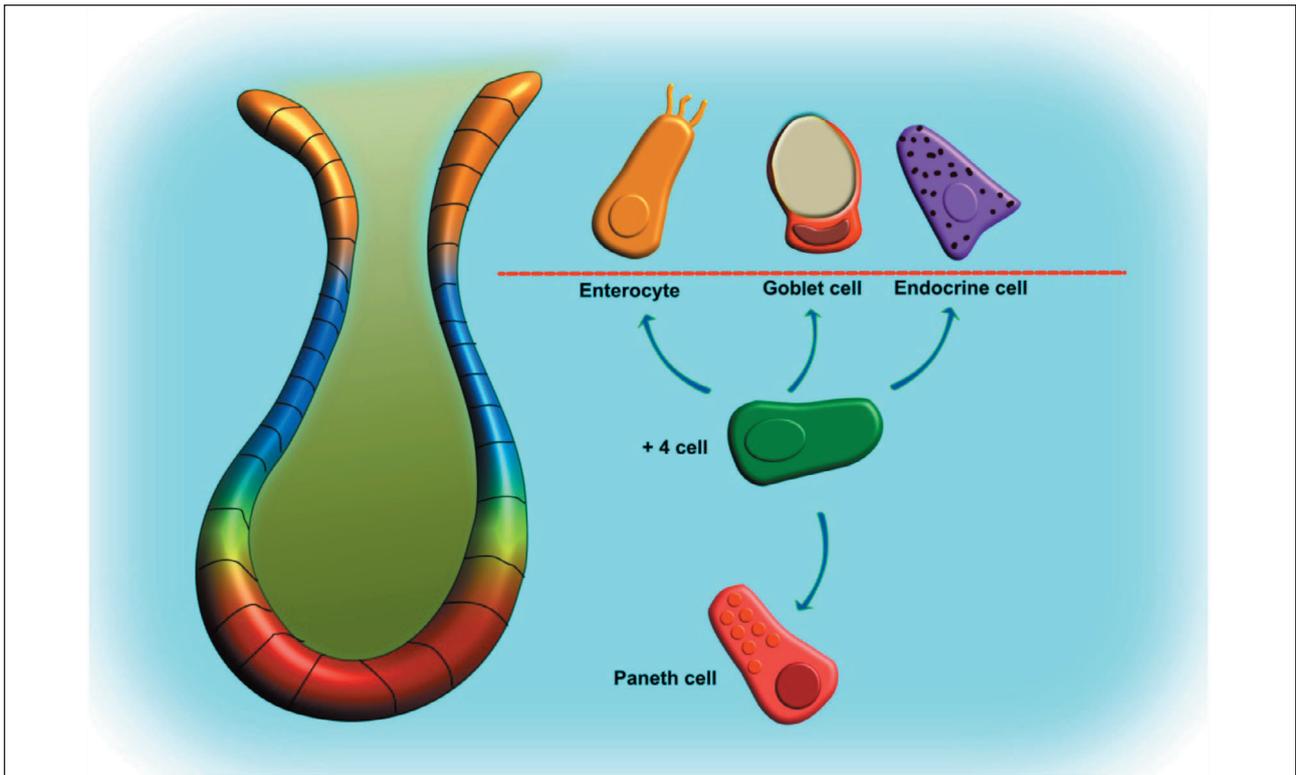
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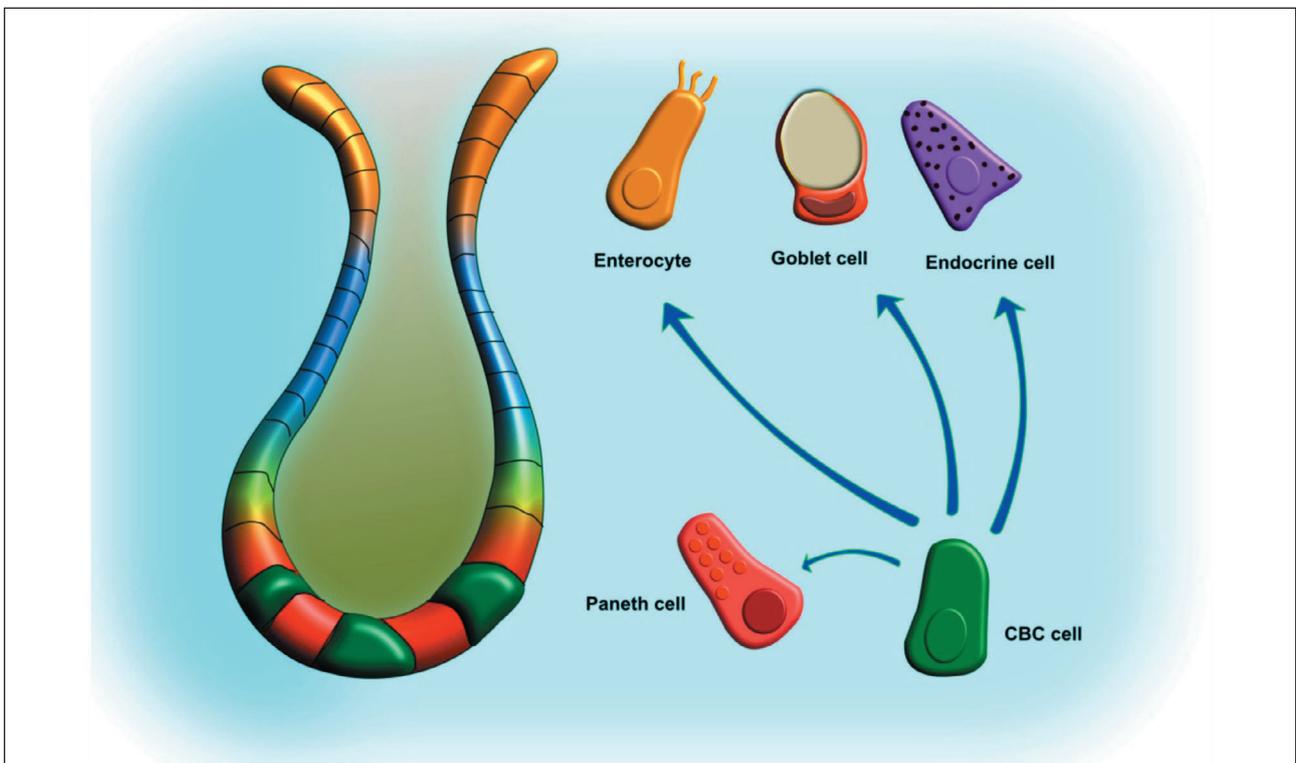
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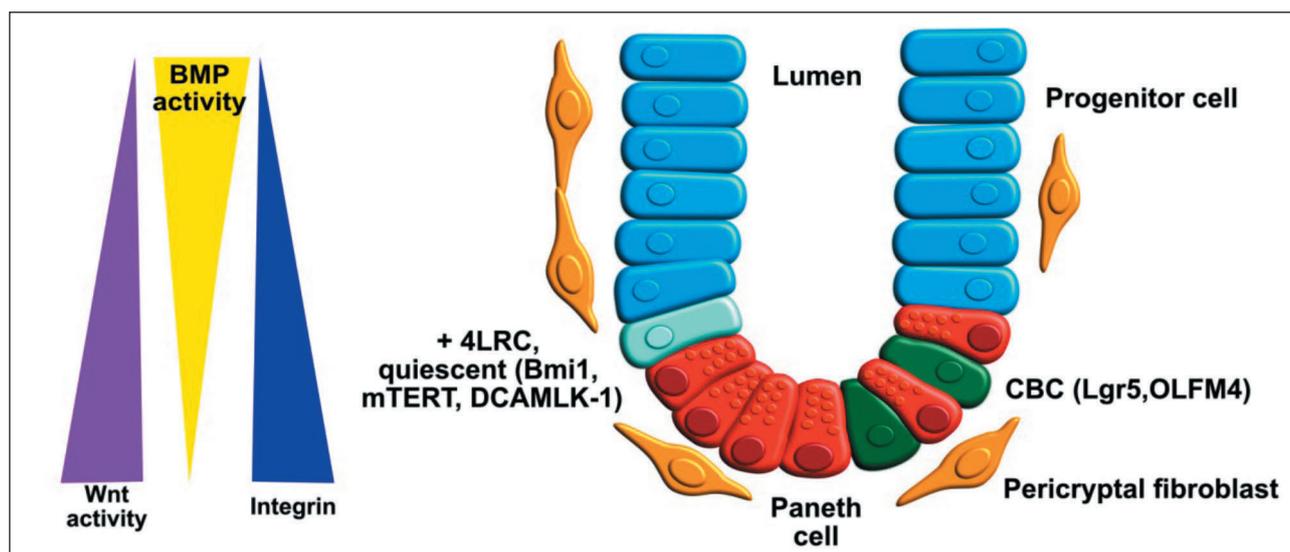
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in the regulation of differentiation of Paneth cells [18]. Wnt signaling is characteristic of the crypt region. The majority of colorectal tumors present mutations in Wnt signaling components, resulting in hyperactivated Wnt signaling, with adenomatous poliposis coli being the most frequently mutated.

Notch signaling plays a pivotal role in controlling the cell fate determination in the TA compartment maintaining that cells at undifferentiated and proliferative states during gut development. Activation of Notch signaling in the intestinal epithelium increases cell proliferation, but inhibition of Notch reduces secretory cells [19, 20].

BMP signaling has a central role as a negative regulator of intestinal epithelial cell proliferation in the intervillus pockets and stimulating the formation of villi [21]. BMP antagonizes Wnt-induced crypt formation and stem cell renewal [22]. Defects in proper BMP-1A signaling result in cell hyperproliferation and can also give rise to colorectal tumorigenesis [22].

Hedgehog, as well as Platelet-Derived Growth Factor A, is expressed by intestinal epithelial cells and target underlying mesenchymal cells that express the relevant receptors and control enterocyte proliferation and villus formation [23].

Mutational activations of these pathways influence gut mucosal growth and could be responsible for intestinal mucosal tumorigenesis [24-27].

MicroRNAs (miRNA) may also modulate ISC proliferation and differentiation.

Stem cell markers in small intestinal crypts

Numerous investigators are involved in studying the location, number, and behavior of ISCs within the base of the intestinal crypts. Several markers are espressed by ISCs. Among these, Leucine-rich repeat-containing G-protein-coupled receptor-5 (Lgr5), Sox9, Prominin-1, DCAMKL-1, EphB2, p-PTEN, p-AKT, Fgfr3, m-TER, and CD44.

Lgr5, also known as G-protein-coupled receptor 49 (GPR49) or G-protein-coupled receptor 67 (GPR67), is a protein encoded by the *LGR5* gene in humans and it is controlled by Wnt signals [28-32]. The latter has been demonstrated in the CBC cells cycling among Paneth cells [33, 34], and it has been shown in colorectal, ovarian, and hepatocellular carcinomas. The precise functional role of Lgr5 in the intestinal epithelium has not yet been cleared; nevertheless, loss of Lgr5 may affect both crypt regeneration and neoplastic transformation.

Musashi-1 (RNA-binding protein Musashi homolog 1) has been proposed as marker for both ISC and early progenitors [35] due to the large number of crypt cells it labels in murine intestinal tissue. In this study the immunohistochemical expression of this protein was observed preferentially in cells toward the base of the crypt, including cells wedged among Paneth cells, in neonatal and adult crypts.

Bmi1 is responsible for maintenance of stem cells in adult. The protein was expressed in cells in the +4 position, but not in the CBC cells, primarily in the duodenum. This may suggest that different stem cell populations may have been characterized.

Crypt Bmi1-positive cells seem to proliferate slowly, in contrast with Lgr5-positive cells, that have fast turnover [36, 37].

Lrig1 (Leucine-rich repeats and immunoglobulin-like domains protein 1) regulates ISC homeostasis by negative control of ErbB signaling [38].

SOX9 is a member of the SOX family of transcription factors and is essential for the differentiation of a small number of cell. In the absence of SOX9, Paneth cells do not form. The lack of SOX9 also leads to increased crypt dimensions and enhanced proliferation in the crypts [39].

Prominin-1 (Prom1) is expressed in a variety of developing and adult tissues. Prom1+ cells are found at the base of crypts in the small intestine, and co-express Lgr5. They generate the entire intestinal epithelium, and are therefore likely to be the small ISC. Prom1 has recently been reported to mark CSC cells of human intestinal tumors that arise frequently as a consequence of aberrant Wingless (WNT) signaling. Prom1 marks stem cells in the adult small intestine, which are susceptible to transformation into tumors retaining a fraction of mutant-Prom1+ tumor cells [40].

Reliable definitive markers would allow for precise identification of ISCs and would be of help in the isolation and *in vitro* manipulation of these cells.

Stem cell therapy in gastrointestinal tract disease

Stem cell therapy has shown promise for the treatment of some diseases characterized by tissue damage with ischemic and inflammatory lesions like inflammatory bowel disease (IBD) and necrotizing enterocolitis (NEC) [41-43]. Native ISCs are responsible for repairing adult intestinal mucosal epithelium in injured tissues and ISCs activity is upregulated following massive intestinal epithelial loss. However, it is likely that the intestinal mucosa of the patients affected by IBD or NEC may have less ability to repair the damage because it harbor lower numbers of functional stem cells. Stem cell therapy for the treatment of those diseases and tissue engineering studies to counter the short bowel syndrome and other diseases probably amenable to stem cell therapy have not yet been adopted for routine use but have already been initiated, and have been met with modest success [42, 43]. Stem cell transplantation and tissue engineered neointestine may provide new functional stem cells to affected patients in order to recover the enteric function, support intestinal restitoration, and alleviate disease symptoms.

Remarkable potential exists in this field of therapy, and new studies are needed to better understand how physicians can supply the patients with new therapies and give them new opportunities.

Declaration of interest

The Authors declare that no conflict of interest exist.

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