Large intestine embryogenesis: Molecular pathways and related disorders (Review)

ANTONIOS KOSTOUROS[1*], IOANNIS KOLIARAKIS[1*], KONSTANTINOS NATSIS[2], DEMETRIOS A. SPANDIDOS[3], ARISTIDIS TSATSAKIS[4] and JOHN TSIAOUSSIS[1]

[1]Laboratory of Anatomy-Histology-Embryology, Medical School, University of Crete, 71110 Heraklion; [2]Department of Anatomy and Surgical Anatomy, Medical School, Aristotle University of Thessaloniki, 54124 Thessaloniki; [3]Laboratory of Clinical Virology, Medical School, University of Crete, 71003 Heraklion; [4]Laboratory of Toxicology, Medical School, University of Crete, 71409 Heraklion, Greece

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Abstract. The large intestine, part of the gastrointestinal tract (GI), is composed of all three germ layers, namely the endoderm, the mesoderm and the ectoderm, forming the epithelium, the smooth muscle layers and the enteric nervous system, respectively. Since gastrulation, these layers develop simultaneously during embryogenesis, signaling to each other continuously until adult age. Two invaginations, the anterior intestinal portal (AIP) and the caudal/posterior intestinal portal (CIP), elongate and fuse, creating the primitive gut tube, which is then patterned along the antero-posterior (AP) axis and the radial (RAD) axis in the context of left-right (LR) asymmetry. These events lead to the formation of three distinct regions, the foregut, midgut and hindgut. All the above-mentioned phenomena are under strict control from various molecular pathways, which are critical for the normal intestinal development and function. Specifically, the intestinal epithelium constitutes a constantly developing tissue, deriving from the progenitor stem cells at the bottom of the intestinal crypt. Epithelial differentiation strongly depends on the cross-talk with the adjacent mesoderm. Major molecular pathways that are implicated in the embryogenesis of the large intestine include the canonical and non-canonical wingless-related integration site (Wnt), bone morphogenetic protein (BMP), Notch and hedgehog systems. The aberrant regulation of these pathways inevitably leads to several intestinal malformation syndromes, such as atresia, stenosis, or agangliosis. Novel theories, involving the regulation and homeostasis of intestinal stem cells, suggest an embryological basis for the pathogenesis of colorectal cancer (CRC). Thus, the present review article summarizes the diverse roles of these molecular factors in intestinal embryogenesis and related disorders.

Correspondence to: Professor John Tsiaoussis, Laboratory of Anatomy-Histology-Embryology, Medical School, University of Crete, Andrea Kalokerinou 13, Voutes, 71110 Heraklion, Greece
E-mail: tsiaoussis@uoc.gr

*Contributed equally

Abbreviations: AIP, anterior intestinal portal; AP, antero-posterior; BMP, bone morphogenetic protein; CIP, caudal intestinal portal; CRC, colorectal cancer; CSCs, cancer stem cells; ENCCs, enteric neural crest cells; ENS, enteric nervous system; FGF, fibroblast growth factor; Fox, forkhead-box; Hox, homeobox; HSCR, Hirschsprung disease; LR, left-right; PHOX, paired-like homeobox; RAD, radial; Shh, sonic hedgehog; Sox, SRY-related HMG-box; Tbx, T-box; TGF-β, transforming growth factor-β; Wnt, wingless-related integration site

Key words: embryology, large intestine, molecular pathway, pathogenesis, cancer, stem cells

Contents

1. Introduction

The mature large intestine is composed of the cecum, colon and rectum. Embryologically, the large intestine is a part of the developing gastrointestinal (GI) tract and shares the same progenitor tissues with the other organs of the GI tract, as it arises during the development of the endoderm. Later during embryogenesis, it incorporates tissue from all three germ cell layers of the trilaminar embryo (1). The epithelium and associated glands derive from the endoderm, while mesenteries, connective tissues, smooth muscle and blood vessels come

from mesoderm, and the intrinsic and extrinsic innervation originate from ectoderm. In order to achieve functional and structural harmony, excellent molecular tissue crosstalk is required during the development of the large intestine (2).

During embryological development, several factors can derail the normal sequence of events which normally lead to the formation of an intact and functioning GI tract. Usually, the disruption of gut tube morphogenesis occurs due to problems in specification and maintenance (3). Some of the developmental colonic abnormalities, which embryos may experience, are atresia, stenosis, duplication, situs inversus, intestinal malrotation and intestinal aganglionosis, malformations of cecum, persistent colonic mesentery and other rare conditions. There is also evidence to suggest a possible connection between colon embryogenesis and carcinogenesis (4).

Hence, in the present review article summarizes the current knowledge regarding the embryology of the large intestine, focusing on the responsible molecular mechanisms along with their disturbances.

2. Overview of gastrointestinal embryogenesis

Initially, the GI tract, which emerges from the endoderm during gastrulation (week 3), extends from the buccopharyngeal membrane to the cloacal membrane. During and immediately following gastrulation, the development of the gut tube occurs in simultaneity with the turning and folding movements of the embryo (1). Thus, three regions begin to form in the sagittal plane, the foregut cephalically in the head fold, hindgut caudally with its allantoic outgrowth and midgut in the middle. Initially, definitive endoderm invaginates at a cranial and a caudal region forming the anterior intestinal portal (AIP) and the caudal intestinal portal (CIP), respectively. As a consequence, the two ends of the GI system are formed. AIP and CIP extend toward one another and the lateral endoderm of midgut folds ventrally to form a sealed gut tube. At the same time, the subjacent splanchnic mesenchyme grows around the endoderm and differentiates to smooth muscle (2). After week 4, the foregut, midgut and hindgut are craniocaudally discernible and they evolve into the different compartments of the GI tract. These three divisions are later distinguished by their different arterial supply. The GI epithelial cells proliferate and obliterate the gut lumen by week 6. By week 8, the central cells degenerate, and the tube is thus again patent.

The parts of the large intestine derive from the midgut and hindgut. The midgut is formed during week 4, as the embryo laterally folds and the pocket of the yolk sac protrudes ventrally. The yolk sac continues to communicate with the primitive gut through the omphalomesenteric duct (vitelline duct). The vitelline duct normally obliterates later, usually before day 36 (5). The arterial supply of the midgut comes from the superior mesenteric artery and it is drained by the corresponding venous and lymphatic vessels. The main GI tract organs which form between weeks 6 and 10 from the midgut are the distal duodenum, jejunum, ileum, appendix, cecum, ascending colon and the proximal two thirds of the transverse colon. The mesenteries of the GI tract are generated from the common dorsal mesentery, while the ventral mesentery participates in the lesser omentum and falciform ligament. During embryological development, much of the midgut herniates at the umbilicus externally to the abdomen, providing a potential position of rotation, which must occur in order to place the GI tract in the correct abdominal position with its associated mesentery (6). Between weeks 5 and 8, the midgut elongates inside its mesentery and forms loops. As a result, a normal umbilical herniation of the midgut occurs, as the midgut loop gradually protrudes through the umbilical ring. At week 8, the total intraumbilical loop rotates counterclockwise 90˚ and positions the midgut along the horizontal plane. By 10 weeks, the abdomen has developmentally enlarged sufficiently so that the entire midgut can be accommodated inside it. Following a further 180˚ counterclockwise rotation around the superior mesenteric artery, the small intestine returns to the abdominal cavity. Concurrently, the large intestine follows its rotation and also moves 180˚ counterclockwise. Following the return of midgut in the abdomen, the mesenteries of cecum and ascending colon fix to the dorsal wall, making these parts immobile (6).

The hindgut initially consists of the cloaca, which later gets segregated by a septum to a dorsal GI compartment and a ventral urogenital compartment (7). Its blood supply mainly comes from inferior mesenteric artery, with corresponding venous and lymphatic drainage. The hindgut later differentiates to urinary epithelium, distal one third of transverse colon, descending colon, sigmoid colon, rectum and superior anus. The transverse colon and sigmoid colon maintain a mobile mesentery, whereas the descending colon becomes immobile as its mesentery fixes to the dorsal wall. As regards the rectum, the upper third is intraperitoneal, the middle third retroperitoneal, whereas the lower third is infraperitoneal along with the superior anus (8).

In the large intestine, the nervous system is represented by intrinsic and extrinsic innervation. The enteric nervous system (ENS), which is the intrinsic innervation, originates from cells of the vagal neural crest migrating into and across the wall of the GI tract (9). The ENS is considered as part of the autonomous nervous system (10,11), and it is composed of two plexuses; the external myenteric plexus of Auerbach between the circular and the longitudinal muscular layers across the entire length of the GI tract, and the internal submucosal plexus, which does not exist in the esophagus and principally includes Meissner's and secondly Schabadasch's plexus (12). Although Schabadasch's plexus is described as a part of the ENS, placed between Meissner's and Auerbach's plexuses, there is a lack of information regarding its embryological formation. The extrinsic innervation of the large intestine is mediated through sympathetic nerves, originating from T5 to L2 levels, and parasympathetic nerves from the dorsal vagus complex and pelvic nerves S2-S4. The major events during gut embryogenesis are summarized in Table I.

3. Early development of the gut tube

The gut consists of two types of tissue in a tubular arrangement. The outer layer(s) of the tube is mainly smooth muscle derived from lateral plate splanchnic mesoderm, while the inner lining is derived from endoderm in the majority of the gut epithelium. On the contrary, the epithelium of the most cephalic (mouth) and caudal (anus) regions of the gut is derived from ectoderm (2).

Table I. Major embryological events during gut development.

Embryonic week	Developmental stage
Week 3	- Gastrulation
	- Primitive gut tube formation
	- Elongation and invagination of endoderm anteriorly, caudally, and ventrally
	- Development of splanchnic mesenchyme around endoderm
Week 4	- Discernibility of foregut, midgut and hindgut
	- Buccopharyngeal membrane resorption
	- Invasion of foregut by enteric neural crest cells
Week 6	- Obliteration of gut lumen
	- Initiation of midgut herniation through the umbilical ring
Week 7	- Obliteration of vitelline duct
	- Completion of enteric neural crest cell migration
Week 8	- Recanalization of gut tube
	- Rotation of intraumbilical loop 90˚ counterclockwise
Week 9	- Initiation of villus formation
Week 10	- Rotation of midgut 180˚ clockwise back to the peritoneal cavity
Week 11	- Development of circular and longitudinal smooth muscle layers
Week 12	- Initiation of crypt formation
Week 14	- Formation of mucosal muscle layer
Week 24	- Development of intestinal absorption function
Week 32	- Fetal intestinal absorption equal to adult levels

Gastrulation begins at the 7th day of embryonal development, when the primitive streak is formed in the pluripotent epiblast layer. The endoderm and mesoderm are derived from the former mesendoderm, through the transforming growth factor-β (TGF-β) family member Nodal (13) and possibly wingless-related integration site (Wnt) signaling (14,15). Depending on the intensity of Nodal signaling, the mesendodermal cells differentiate to the endoderm, whereas lower Nodal signaling induces differentiation towards mesoderm (13,16-19). Transcriptional factors which are members of the T-box, SRY-related HMG-box (Sox), Mix, GATA and forkhead-box (Fox)-A families have been found to regulate the promotion of Nodal-induced endoderm formation in all vertebrate species (20-24). Activin is another TGF-β family member, which binds to the same receptors with Nodal and can mimic the role of Nodal in the induction of mesendodermal differentiation towards the endoderm (25,26).

The anterior definitive endoderm is created by the first cells which emerge through the primitive streak and migrate anteriorly (27,28). On the contrary, the endoderm of the midgut and hindgut are derived from the mid-streak stage of gastrulation and the entire definitive endoderm is completely formed before somite formation (29,30). It is noteworthy that the definitive endoderm intercalates with the existing extraembryonic visceral endoderm (31), mostly in hindgut final endoderm (35%), possibly masking genetic disorders of its development.

The initial biological events leading to AIP and CIP formation following gastrulation have not yet been determined. The AIP is created by cells of cephalic endoderm, while the CIP forms by caudal endodermal cells (29,32,33). In the beginning, endodermal cells from the cephalic and caudal pits invaginate and form pockets, further elongating until they meet each other and merge. Simultaneously, the lateral endoderm of the midgut ventrally folds and completes the closing of the gut tube (34). A study using *Xenopus* frogs proposed that both the elongation and gut tube formation, require Rho, Rho kinase (ROCK) and myosin II in order to regulate cellular and tissue arrangement (35).

Several factors have been investigated to evaluate their participation in the mechanisms which promote the invaginations. The GATA family of transcriptional factors seems to be implicated in the events of invagination. GATA-4 has been found to be expressed in the very early definitive endoderm of the AIP and is necessary to close the body wall. GATA-$4^{-/-}$ embryos develop a malformed AIP and therefore, no foregut. They also evolve without the normal placement of the yolk sac due to the defective lateral-ventral body folding and the abnormal AIP. However, GATA-$4^{-/-}$ embryos maintain the anterior endoderm, indicating that other factors are crucial for its development (36).

There is evidence to indicate that in mice, the formation and elongation of the gut tube is guided by the regulation of the ultimate extension movements of the embryo. A Kruppel associated box (KRAB) zinc-finger protein, Chato, directs body elongation of all three germ layers. Chato has been shown to affect endodermal elongation, and embryos lacking Chato experience failure of endodermal elongation and unsuccessful gut tube closure (37). Contrary to Chato, dapper (Dact)-1 is implicated in gut tube morphogenesis via modulating the non-canonical Wnt/planar cell polarity (PCP) signaling pathway. Dact-$1^{-/-}$ mice experience impaired posterior development with the failure of the hindgut endoderm to form CIP,

failure of the ventral endodermal folding and failure to form cloaca or hindgut (38).

The molecular mechanisms controlling the development of the mesoderm are better understood in comparison with those regarding the endoderm, since their interaction is crucial for the formation of the gut tube (2).

It is a fact that cultures of endodermal cells from the foregut cannot differentiate without co-culturing with mesodermal tissues (39). There is a developmental window before which the differentiation of primitive endoderm relies on the antero-posterior (AP) position of its subjacent mesoderm (39). After this window, the morphologically undifferentiated primitive GI endoderm is committed to develop into regionally specific epithelium (40). Furthermore, studies have confirmed that the endoderm can also alter the differentiation of mesoderm. When somatic, non-gut mesoderm is co-cultured with gut endoderm, it is directed to differentiate towards smooth muscle rather than skeletal muscle and this is confirmed by histology and by tracing the expression of mesodermal proteins, e.g., tenascin (41) and of smooth muscle actin (42).

The majority of endodermal gut regions exhibit morphological and cellular plasticity to the influence of mesoderm except for the midgut, where the endodermal cells are more autonomous (43,44). Some molecular events which coordinate the endodermal-mesodermal co-development have been described. Sonic hedgehog (Shh), a vertebrate homologue of *Drosophila* hedgehog (hh), encodes a signaling molecule which participates in the development of limbs (45), somites (46) and neural tube (47). Shh is expressed in the endoderm of the GI tract and its derivatives (48) it has been hypothesized to initiate the early endodermally-derived inductive signal for the gut morphogenesis. Initially, it is expressed only in AIP and CIP even before the beginning of invaginations (49). However, it has been proven that this is not the molecule which signals for the beginning of invaginations (50), as $Shh^{-/-}$ mice develop a GI tract even with major abnormalities in the foregut, such as malformed esophagus with wider lumen and without a surrounding mesoderm (51). Therefore, Shh endodermal signal towards mesoderm probably mediates development, recruitment and other aspects of mesoderm in foregut. It has been confirmed that Shh receptors exist only in the mesodermal part of the GI tract (52), whereas the overexpression of Shh in the primitive gut leads to the overdevelopment of the mesoderm rather than endoderm (44).

Wherever the endoderm expresses Shh, the subjacent mesenchymal mesoderm expresses a homologue of *Drosophila*'s decapentaplegic (dpp) (48). Bone morphogenetic protein (BMP)-2 and BMP-4 are the two dpp homologs which are expressed in the vertebrate gut. Even in the primitive gut, before invagination becomes apparent, by the time Shh expression is traceable in the CIP region, BMP-4 is expressed in the closely associated mesenchymal mesoderm (49). Shh has been proven to induce BMP-4 in the splanchnic mesoderm even ectopically (44,49,52).

BMP-4 may negatively regulate the growth and hypertrophy of smooth muscle or it may facilitate differentiation to smooth muscle. At a later developmental stage, when the smooth muscle layers of the gut have already formed, BMP-4 is expressed in the submucosa, which is the mesodermal tissue subjacent to the Shh expressing endoderm. On the contrary, the expression of BMP-4 cannot be traced in the differentiated smooth muscle region of mesoderm, which does not have direct contact with endoderm. It is noteworthy that both Shh expression and BMP-4 do not induce smooth muscle differentiation of mesoderm. Shh has even been found to reduce the development of smooth muscle proteins in explant cultures (52).

The role of BMP-2 in the development of a functional gut has been evaluated and seems to be mainly implicated in its enervation. BMP-2 is expressed in gut mesoderm and has been found to promote the maturation of enteric neurons (53). Nevertheless, its overexpression deregulates the contribution of other crucial factors on the incorporation of neural cells in gut, including colon, impeding the survival of neural cells in the gut (54).

4. Molecular control of the antero-posterior (AP) pattern

The GI tract is patterned into distinct AP compartments, a fact that can be validated by the expression of hematopoietically-expressed homeobox (Hhex), Fox-A2 and Sox-2 anteriorly and caudal-related homeobox (Cdx)-1,2,4 posteriorly (30,55-57). Even following gastrulation, the endoderm continues to receive signals mainly from mesoderm, according to which it gets further patterned (57). The signaling continues in each developmental step, thus being vulnerable to possible alterations and/or disorders. Some of the most important molecular pathways which mediate the patterning of the AP axis of the endoderm include fibroblast growth factor (FGF), Wnt, BMP and retinoic acid (RA) signaling (10,58-61).

Role of FGF. The exposure of human embryonal stem cells (hESCs) to a particular concentration of 1.1 ng/ml of FGF-4 augments their pancreatic and duodenal homeobox (Pdx)-1 expression, which is primarily induced by RA signaling. Moreover, FGF-4 and RA possibly cooperate to increase Cdx-2 expression of the posterior gut endoderm (62). It has also been demonstrated that as far as the concentration of added FGF-2 increases, endodermal hESCs are patterned towards either an anterior phenotype, or a posterior phenotype, respectively to the increase of FGF-2 concentration (63). Finally, FGF and Wnt collaborate to direct endodermal hESCs towards a hindgut Cdx-2-positive epithelial phenotype (64). Cdx-2 is recognized as one of the most conserved markers of hindgut, while the majority of the colon is derived from it.

While the endodermally-derived epithelium develops, FGF-9 is required to be expressed by the epithelium so as to signal towards the surrounding mesenchyme and harmonically direct it to proliferate and elongate according to the respective growth of its underlying epithelium (65). In response, the mesenchyme simultaneously expresses FGF-10, which signals back to the epithelium through FGF receptor (FGFR)2b and directs it to proliferate and form the cecal budding. However, FGF-10 does not induce the specific differentiation of the cecal epithelium (66-68).

Role of Cdx. The endoderm, indeed, possess intrinsic molecular pathways, which wait for the time to respond and guide endoderm to its proper differentiation and functional specification in accordance with extrinsic inductive signals.

Pertaining to the colon, the hindgut endoderm maintains a fixed epithelial identity following a certain stage of development. As a consequence, mesoderm can only limitedly alter the AP developmental pattern of endoderm prior to cytodifferentiation (69).

One of the most significant intrinsic molecular factors for hindgut intestinal differentiation is Cdx-2, which is a member of the ParaHox gene cluster (70,71). It has been demonstrated that the lack of Cdx-1 or Cdx-4 results in a non-intestinal phenotype (72). Furthermore, if Cdx-2 loss-of-function is imposed, the endodermal differentiation is disoriented, the intestinal phenotype is not rescued and the gut epithelium is altered to esophageal epithelium. It is interesting that, even though Cdx-2 loss-of-function leads to a posterior to anterior alteration, the expression of other important factors of the AP differentiation, such as homeobox (Hox) genes, Pdx-1 and Barx-1 were unaffected (70). Therefore, Cdx-2 indeed is a key molecular factor and it is required for both the establishment and maintenance of the posterior endodermal phenotype. The combined action of the most important extrinsic factors, Wnt, FGF, BMP and RA at each developmental stage may be necessary to induce the proper Cdx-2 activity (73-75). T-cell factor (Tcf)-1 and Tcf-4$^{-/-}$ mice develop the loss of the most caudal hindgut region and alteration in the differentiation of the duodenum, which, instead of Cdx-2, express Sox-2 which is a stomach marker (76). This evidence appears to be very similar to the results of the Cdx-2 loss-of-function (71).

Role of Hox genes. The molecular pathways which control the overall body plan are probably implicated in the AP pattern of the gut as well. There are indications that Hox genes, which participate in the patterning of the total body plan, initially were meant to pattern the gut. The abdominal (Abd)-B-like Hox genes are expressed in the mesoderm of the posterior midgut and hindgut, whereas clusters A and D of these genes are regionally expressed, defining morphological landmarks of the mid- and hindgut (49,77). It has been assumed that Hox genes are important in setting up limits between major AP regions, such us sphincters. Nevertheless, Hox genes mutations seem to provoke only minor defects in the developmental of endoderm (78-80).

The most posterior region of the gut, the cloaca is the tissue that later evolves to both the anorectum and urogenital opening. In humans, a missense mutation in Hox-A13 results in protein truncation and finally in a syndrome that includes a genital phenotype (81). Elaborating on the role of Hox genes, it seems that they also participate in the patterning of the gut epithelium. The misexpression of Hox-D13 in the chick midgut mesoderm induces a hindgut differentiation of the midgut mesoderm epithelium. Shh expressed in the endoderm has been identified as an activator of Abd-b like Hox genes (49), while Shh also induces Hox genes in the limb (45). Thus, Shh may be a general inducer of Hox genes.

Role of Wnt pathways. Another very important system of signaling for the AP pattern is the Wnt. Wnt genes encode a variety of molecules which signal through the Frizzled (Fz) family of cell surface receptors, activating several intracellular pathways, such as the Wnt/β-catenin canonical pathway, the Wnt/Ca^{2+} pathway and the planar polarity pathway (58,82). Of the Wnt ligands, Fz7, Fz8, Fz9, Wnt5b, Wnt 6 and Wnt 14 are traced during the early or late stages of gut development (83,84). Of the Wnt antagonists, Frizzled-related protein (FRZ)-b1 and secreted frizzled-related protein (sFRP)-2 are expressed in the developing gut. Of the downstream targets of the canonical pathway, Tcf-4 and Tcf-7L2 are expressed in the colon (83,85). While the embryonal development evolves, various Wnt ligands, antagonists and downstream molecules are detected. A combination of different proteins is found in very distinct regions along the AP axis of the gut tube, in correspondence with the boundaries of the future organs of the GI tract (83). For instance, lymphoid enhancer-binding factor (Lef)-1, Fz1 and Fz8 are expressed in the duodenum and large intestine.

The canonical Wnt pathway. Along the canonical pathway, which is the most extensively studied of the Wnt pathways, β-catenin translocates to the nucleus and it associates with members of the Tcf/Lef family of high mobility group (HMG) transcriptional factors to transduce the Wnt signal (86). If Wnt is absent or insufficient, β-catenin becomes phosphorylated and is degraded by β-transducin repeats-containing protein (β-TrCP), an E3 ubiquitin ligase (87). This phosphorylation requires glycogen synthase kinase (GSK)-3β, axin, and adenomatous polyposis coli (APC) (86,88). The active, functioning canonical Wnt pathway allows β-catenin to escape phosphorylation and degradation (89).

The expression profiles of molecules of the canonical Wnt pathway define domains that give rise to the duodenum, ceca and large intestine, whereas non-canonical pathway members define regions which will become the posterior small intestine and cecum. To elaborate, there is evidence that the Wnt canonical signaling pathway guides the AP patterning of the gut by directly augmenting the expression of Cdx-2. However, another study demonstrated that Cdx-2 expression was also decreased after the withdrawal of a Wnt antagonist and the subsequent reactivation of Wnt signaling, which seems a bit confusing (90). Therefore, the canonical and non-canonical Wnt pathways may define distinct domains in the AP axis and they may have different functions in regions of the gut where they overlap, such as the cecum (83).

A number of disorders can deregulate the dynamic equilibrium between the activation and degradation of β-catenin and of the activity of the overall canonical pathway. Axin mutations prevent axin from binding to β-catenin, blocking the deactivation of β-catenin by APC. β-catenin mutations can also rescue β-catenin from phosphorylation for degradation by β-TrCP (87). As a consequence of the above mutations, β-catenin levels remain stable and can continue the activation of the canonical Wnt pathway endlessly (91).

Lef-1 is a transcriptional mediator in canonical Wnt pathway, but is not normally expressed in the colon (92). Lef-1$^{-/-}$ chicks exhibit stenosis of the cecal lumen due to the overproliferation of epithelial cells. Tcf-1 is a downstream target of Tcf-4/β-catenin (93) Tcf-1 and Tcf-4$^{-/-}$ mice exhibit a lack of crucial caudal structures, while the main defect in these mice is localized in the endoderm, contrary to the majority of canonical Wnt pathway disorders which primarily disrupt the mesoderm. These mice do not form a CIP, their hindgut endoderm does not express Fox-A1 and Sox-17 and they end up with

an open midgut and a total lack of hindgut structures related to the complete absence of Shh expression (76). The maintenance of the stem cell compartment is potentially mediated by Cdx-1, which is a downstream target of Tcf-4 and is expressed in the crypts of the intestinal epithelium (94). Tcf-4 and Lef-1 are evidently important for the adjustment of the balance between proliferation and differentiation in the developing gut.

The lack of Wnt3a, Wnt5a, low-density lipoprotein receptor-related protein (LRP)-6 or Tcf-1/Lef-1 all lead to mesodermally mediated posterior developmental disorders (95,96).

Non-canonical pathways. At the non-canonical planar cell polarity or Wnt/PCP pathway, ROCK and the Jun-N-terminal kinase (JNK) are activated (97,98) and participate in the direction of the cytoskeletal organization and of the epithelial cell polarization (99). This pathway is also implicated in the extension movements of the mesoderm during gastrulation, in which Wnt11 and Fz7 ligands are required (100).

A second non-canonical Wnt pathway, the Wnt/Ca^{2+} pathway, is implicated in the extension movements of the mesoderm and in the ventral development of the embryo (101,102). It acts by the release of intracellular Ca^{2+} following the activation of phospholipase C, protein kinase C and calmodulin-dependent kinase II. Wnt5a, which participates in the canonical pathway as well, and Fz2 are necessary for the intracellular release of Ca^{2+} (103).

Not only the activation, but also the deactivation of Wnt signaling is crucial for the patterning of the total body plan (104,105). Antagonists of Wnt, such as the sFRP, block the activity of Wnt ligands with relative specificity. For instance, FRZ-b1 and sFRP-2 inhibit Wnt1, but only FRZ-b1 can block Wnt8 and only sFRP-2 can block Wnt4 (105), while all Wnt1, 4 and 8 are ligands in the canonical pathway (106). It has also been observed that in regions with the absence of sFRP-1, the Tcf-4 transcript is found, proposing a positive link between the antagonist and the downstream target of Wnt.

After the gut tube is fully formed, the intestine commences to lengthen. Wnt5a seems to be implicated in this elongation via mediating in the non-canonical Wnt signaling pathway. $Wnt5a^{-/-}$ mice develop a 63% shorter intestine than Wnt efficient mice (107). It is noteworthy that mice that have ineffective proteins of the sFRP family, which normally antagonize Wnt5a, also exhibit a shorter intestinal length (108). This evidence suggests that the development of proper intestinal length requires the balanced regulation of Wnt signaling, and neither the its hyperactivation nor hypoactivation.

5. Molecular control of the left-right (LR) pattern

The molecular events which are implicated in the configuration of the LR pattern are among the most conserved events among all species (109).

Two crucial molecules which are linked to the creation of LR asymmetry are Shh and Activin (110). The left side of the embryo restrictedly expresses Shh, whereas Activin is expressed only in the left side. Shh expression leads to a cascade of unilateral expression of factors, such as Nodal, paired-like homeodomain transcription factor (Pitx)-2, bagpipe homeobox homolog (Bapx)-1 and FGF-8 (111-113). Bilateral Shh expression would lead to the randomization of the situs of each organ independently (112). Pertaining to the gut, the levo-situs is defined by the same left-sided factors, such as Shh, Pitx-2 and Bapx-1, whereas the bilateral expression of these molecules would lead to heterotaxy syndromes (112).

Herniation and rotation. The primary midgut loop, which is formed during midgut herniation into the umbilical cord at week 5, as mentioned before, contains only one part of the colon, the cecum, which is part of the 4th secondary ileocecal loop of the distal limb (114). The colon develops 2-fold at a slower rate than the small intestine, in accordance with its dorsal mesentery and does not require to loop (115). Before the resolution of the hernia, the intra-abdominal part of colon and its dorsal mesentery is sagittally positioned in the midline of abdomen. Between weeks 8.5 and 9.5 of gestation, the development rate of both small and large intestine significantly decreases (115), whereas the body axis and wall growth create enough space to drive midgut back inside abdomen (116). As the midgut returns inside the abdomen, the duodenum is placed dorsocaudally to the superior mesenteric artery (SMA) at the left side and jejunum occupies most of the left side. The ileocecal loop is the last to return inside the abdomen at week 9.5, and becomes positioned initially ventrally at the midline and half a week later it can be found in the right side (115).

The ascending and proximal transverse colon, which are also suspended by the midgut mesentery, initially have a ventrocranial position relative to SMA, and move inwards following the movements of the intra-abdominally returning small intestine and maintain their continuation with cecum, thus occupying a right sided position. In the meantime, at week 6 the last part of the midgut, the future distal transverse colon, is sagittally continuous with hindgut and they are hardly discernible. At week 8, the transition of the midgut to the hindgut, as it can be recognized by the overlap of perfusion by branches of both SMA and inferior mesenteric artery (IMA), begins to lift and move leftward to form the splenic bend. As gestation proceeds, the distal part of this transition extends downward and forms the descending colon. At week 10, while the cecum is positioned cranioventrally relative to small intestine and subhepatically due to the relatively large size of liver, proximal colon runs its course obliquely towards the splenic bend (114,117). Between weeks 10 and 20, while the relative size of the liver decreases (118), the hepatic bend develops, the ascending colon extends downward at the right abdominal side and the cecum follows and occupies its final position in the right iliac fossa (117).

The molecular and structural interactions that promote the phenomenon of rotation, which are crucial for the proper positioning of the colon, have not been investigated in detail; however, there is evidence to indicate that the dorsal mesentery of the midgut loop presents molecular and architectural left-right asymmetry. The mesenchymal cells of the left side of the mesentery are more condense than those of the right side, whereas the midgut epithelium of the left side is columnar, while the right-side epithelium is cuboidal. Therefore, there seems to be a greater proliferation of the mesenchyme and epithelium on the left side, which condenses the cellular and non-cellular structures creating mechanical forces which tilts the mesentery and the midgut loop counterclockwise (119).

To elaborate on the molecular factors, the transcriptional factor Pitx-2, which can be activated by Nodal, is restrictedly expressed in the left side of the dorsal mesentery in its whole dorsal-ventral extent (111). This may be one of the results of the leftward flow, which is generated by the asymmetric beating of nodal cilia (63,120,121). Concurrently, islet (Isl)-1, a LIM homeodomain-containing transcriptional factor, is exclusively expressed in the left side of the mesentery at the time of rotation, whereas T-box (Tbx)-18 factor is expressed at higher levels in the right side than the left side of the midgut loop mesentery (119,122).

Shroom3 and N-cadherin have been proposed as downstream targets of Pitx-2, which mediate the cellular shape changes that characterize the left-right asymmetry (123). *In silico* has analysis established that N-cadherin is exclusively expressed in the left side of the mesentery and it fosters the asymmetry of the extracellular matrix (ECM), being partly responsible for the mesenchymal left-right side asymmetry. The mesenchymal asymmetry also exists owing to different cell to cell adhesion (124). Another possible Pitx-2 downstream target is dishevelled associated activator of morphogenesis (Daam)-2 and it is activated in the dorsal mesentery both directly and indirectly by Pitx-2. The indirect activation is mediated by Wnt signaling (125).

Disorders: Misexpression of key factors. Either the ectopic bilateral expression of Nodal, which is known to activate Pitx-2, or the ectopic expression of Pitx-2 itself bilaterally in the splanchnic mesoderm forces a symmetrical bilateral expression of Isl-1 and a loss of right sided Tbx-18 expression. Isl-1 probably augments Pitx-2 expression, as the ectopic expression of Isl-1 in the right side of the mesentery also leads to bilateral Pitx-2 expression and the loss of right-sided Tbx-18 expression. The ectopic expression of either of the above-mentioned factors, Nodal, Pitx-2 or Isl-1, lead to the disruption of the left-right asymmetry of the dorsal mesentery, creating a bilateral symmetry with the replacement of the cuboidal cells of the right side by columnar cells and an increase in mesenchymal density of the right side. Pitx-$2^{-/-}$ embryos do not express Isl-1; they express Tbx-18 bilaterally and present bilateral symmetry as the left side acquires the structural characteristics of the right side (119). As a conclusion, Pitx-2 and Isl-1 expression seem to be significant in creating the dynamic force, which directs the initial events of midgut rotation.

Disorders: Situs inversus. In situs inversus, either abdominus or totalis, beyond the other clinical manifestations, the organs of abdomen swap sides. Therefore, pertaining to the colon, the cecum, appendix and ascending part are located in the left side, whereas the descending and sigmoid colon are on the right side. Relatively rare complications of situs inversus are cecal volvulus and intestinal atresia (126). This syndrome occurs due to problems of the nodal function after the stage of 3 somites if there are mutations or defect in genes, such as inversin, kinesin family member (KIF)3B, Dishevelled (Dvl), polycystin (Pkd)-2 and KIF3A. The improper function of inversin reduces the forwarding effectiveness of the cilial movement (127,128), and the lack of KIF3B impairs ciliogenesis, resulting in prenatal death and LR asymmetry (129). Furthermore, Dvl-1, 2 and 3 intervene in both the canonical and non-canonical Wnt pathways. The non-canonical Wnt/PCP pathway is crucial for the proper polarization and function of the node (130) and the malfunction of Dvls leads to the PCP deregulation-mediated randomization of LR asymmetry (131,132). Finally, Pkd-2 or KIF3A loss diminishes the mechanosensory ability of the node, which is necessary to maintain the leftward nodal fluid flow. As a consequence, LR asymmetry distribution randomly occurs (133).

Disorders: Heterotaxy syndromes. Intestinal obstruction, due to cecal volvulus or intestinal atresia, is far more common in heterotaxy syndrome (134). Heterotaxy syndrome differs from situs inversus in that the internal organs are abnormally positioned in the chest and abdomen without some order whereas in situs inversus the order is maintained. Abdominal abnormalities can be present in both of its subgroups, although they are more frequent in the subgroup of isomerism of the left atrial appendage. Intestinal malrotation can derive from failure of the 270˚ counterclockwise rotation of the midgut, whereas intestinal obstruction with the danger of necrosis can occur due to flaws of mesenteric fixation to the dorsal wall (135). Moreover, the cecum does not reach its normal position in the right lower quadrant because the cecal mesentery improperly fuses with posterior parietal peritoneum increasing the hazard of cecal volvulus (136-138). Possible molecular causes of heterotaxy syndromes, affecting normal intestinal embryogenesis, may include insufficiency of Fox-A2, which is known to be a Nodal regulator, and disturbances of the function of FGF-12, RNA binding Fox homolog (RBFox)-1, microRNA (miRNA/miR)-302F and polypeptide N-acetylgalactosaminyltransferase (GALNT)11 (139-141). GALNT11 dysfunction deregulates the mechanisms that ensure harmonic maintenance of left side flow in the node, creating insurmountable obstacles to the proper designing of left-right asymmetry.

6. Mesenteric fixation

The fixation of the colon at the dorsal wall is the least structurally and molecularly understood procedure pertaining to the embryology of the colon. Fixation is a process which occurs only in primates, making it difficult to perform experiments because the available animal models usually are not primates (142). As far as the colon is concerned, at 10 weeks of gestation, directly following the resolution of the umbilical hernia, the ascending and descending colon initially adhere to the dorsal body wall and their dorsal mesentery gradually fuses irreversibly with the parietal peritoneum, which line the internal surface of the abdominal cavity. In such a manner, the cranial part of the ascending colon attaches to the ventral surface of the duodenum and the mesentery extends downwards and leftward, while the greater omentum attaches to the ventral side of the transverse colon and the descending colon mesenteric fusion begins cranially and continues caudally (115,143). The product of fusion, Toldt's fascia or membrane mesenterii propria, contains nerves, blood and lymphatic vessels, lymph nodes and fat tissue (144). Toldt has been the principal investigator of mesenteric fixation and the majority of our knowledge is derived from his observations (144).

The absence of fixation of the colon, termed 'persistent colonic mesentery' or 'mobile colon', normally exists in 20% of infantile autopsies without intestinal malrotation, in 14% of adult autopsies, and in 10% of patient with intestinal volvulus (145).

At 9-10 weeks of gestation, the similar fusion of peritoneal layers seems to exist in the most caudal region of the peritoneal cavity, the prerectal peritoneal pouch, at the transverse level of S3 sacral vertebra. The adhesion and fusion of the peritoneal walls lead to the creation of symphysis that constitutes the rectoprostatic fascia (146,147).

7. Cecum and appendix embryology

The cecum and the appendix derive from the 'bud of cecum' which forms in the midgut just next to the apex of the umbilical herniation at week 6. This bud can be used to recognize the transition of ileum to colon. The cecum changes positions following the rotation of the midgut and the elongation of the ascending colon. The increasing accumulation of meconium inside the cecum is possibly responsible for its increased diameter (148).

The appendix becomes traceable at week 8 of gestation, whereas it may remain thin because it cannot be filled with content due to the existence of mucosal folds in the distal cecum, which confine the flow towards the appendix (148). Lymphatic cells begin to colonize the epithelium of appendix during weeks 14 and 15, positioned directly under the epithelial compartment, which contains relatively fewer goblet cells than the rest of colon. It is noteworthy that the appendix does not possess any lymphatic vessels. Postpartum, while the cecum dislocates laterally, the appendix remains in a more medial position (148).

Cecal and appendical malformations. Possible embryological malformations, which partly or individually implicate the cecum, include non-rotation, malrotation, hyper-rotation, as well as subhepatic, mobile, inverse, retroperitoneal cecum and internal hernias (148). In non-rotation, there is failure of the last 180˚ rotation of the midgut, resulting in the left-side positioning of the whole colon, including the cecum. In malrotation, only the last 90˚ of the midgut rotation do not succeed, placing the ileocecal loop below the pylorus. As an unfortunate consequence, the cecum may become attached to the dorsal body wall with ligaments, which may compress the cecal opening towards the duodenum, leading to ileus (149). In hyper-rotation, the cecum is positioned at the splenic bend of colon either directly or indirectly, due to hyperdescent and travelling through the pelvis and all the way cranially until the left colic flexure (150). In the subhepatic cecum, the elongation of the ascending colon either does not occur or is insufficient, impeding the normal descent of cecum. Therefore, the cecum-appendix complex may be found anywhere from the subhepatic region until the right iliac fossa. The malformation of the mobile cecum occurs after failure of the fixation of the mesentery of ascending colon to the dorsal parietal peritoneum. This situation predisposes to cecal volvulus or malposition of the cecum and may sometimes require urgent surgery for a condition that may resemble acute appendicitis (151,152). The inverse cecum untimely fixes in the subhepatic region and the forthcoming elongation of the ascending colon forces it to bend cranially. In the case of retroperitoneal cecum, a membrane is created, Jackson's membrane, which encloses the cecum and ascending colon and differs from peritoneal adhesions in that it contains blood vessels. The most frequent internal hernia in the cecum, which is the second most usual type of intestinal hernias, is created towards the left paracecal region (148).

The most frequent embryological malformations of the appendix are agenesis and duplication, even though they are relatively rare (148,153).

8. Molecular control of the radial (RAD) pattern

Early obliteration of the lumen. Patterning along the RAD axis is the last to begin chronologically and it continues throughout the life of an organism (154). There are significant indications that, during week 6 of gestation, before epithelial maturation, the GI lumen normally obliterates as a result of epithelial hyperproliferation. In 1900, Julius Tandler observed that, at day 42 of human embryologic development, the epithelium of the duodenal endoderm markedly thickens to such a degree, that the former lumen converts to a solid string without lumen. Between days 44 and 46, duodenum begins to recanalize, while numerous tiny canals are created, which eventually merge into one common lumen (155). However, over the years, it has been difficult to reproduce and investigate the same phenomenon in animal models as neither rats nor mice undergo luminal obliteration of their gut lumen (156).

Recanalization disorders: Atresia. In atresia of the colon, which usually coexist with atresia of other GI compartments, most frequently the duodenum, the mesodermal surrounding and blood supply are absent. Colonic atresia represents approximately 10% of total congenital intestinal atresia cases (157). The categorization system of Louw, is often being utilized even today, even for different sections of intestine rather than duodenum (158). In some theoretical basis, a number of factors have been implicated for this malformation, such as maternal psychiatric conditions, bile secretion problems, intestinal malrotation, mechanical compression and obliterative embryonic conditions.

As far as mechanical compression is concerned, two pathologic entities have been implicated in the development of intestinal atresia, gastroschisis and volvulus. The main hypothesis in both of these conditions is that the limitation of blood flow leads to atresia. Gastroschisis leads to the consequent herniation of intestinal loops and strangulation of their vasculature (159,160). On the other side, intestinal volvulus, either if it is a result of impaired rotation and fixation or after twisting of the intestine around some adhesive band, impedes intestinal blood flow (161-163). Cystic fibrosis has also been epidemiologically related with intestinal atresia without any recognized pathophysiological mechanism (164). However, the vast majority of these atresia cases does not pertain to the colon (161).

Based on the initial hypothesis that the obstruction of blood flow can elicit intestinal atresia, thromboembolic events have also been investigated as possible causes (165). A study established statistical correlation between the presence of factor V Leiden or R353R mutation of factor VII and the development of

intestinal atresia (166). Nevertheless, this scenario is not very convincing, as factor V Leiden is not usually associated with arterial thrombosis (167) and the levels of factor VII are low during gestation due to the insufficiency of vitamin K (168).

Atresias are usually accompanied by developmental disturbances of other organs, such as esophagus, pancreatic duct, bile duct, heart and rectum. All of these structures emerge in the midline and either develop from the endoderm or, in the example of the heart, are significantly affected by the neighboring endoderm. Omphalocele has also been reported as a pathological result of intestinal atresia in association with abdominal wall defects; however, the exact common defective mechanisms remain unknown (169). Nevertheless, some defect early during endodermal development can justify intestinal atresia and the effect on the other organs (170). Disorders of three different molecular complexes have been proven to be able to provoke intestinal obliteration. Firstly, mutations of either FGFR2IIIb or its ligand, FGF-10, which function only in the endoderm, can lead to colonic and duodenal atresia by altering the equilibrium between epithelial apoptosis and proliferation even before any vascular alterations (171,172). In FGFR2IIIb$^{-/-}$ mice, atresia does not occur owing to some epithelial plug inside the lumen. Moreover, in FGFR2IIIb$^{-/-}$ mice with atresia, in which Fox-F1 expression is disrupted, early epithelial apoptosis and total epithelial loss seem to be the core causes of the pathophysiologic event (173). The established Fox-F1 disruption is not the most crucial problem as, even though the presence of exogenous Shh restores Fox-F1 expression that was previously disrupted, it does not alter the phenotype (174,175). Furthermore, in FGFR2IIIb$^{-/-}$ mice, the downregulation of the expression of retinaldehyde dehydrogenase (Raldh)-2 is observed, indicating some alteration of RA signaling (176). However, haploin sufficiency of Raldh-2 in FGFR2IIIb$^{-/-}$ mice diminishes the risk for intestinal atresia (177). Secondly, Hedgehog signaling and its disruption through Shh mutations can also evoke phenotypes of the spectrum of intestinal atresia (178). The role of other molecules which are implicated in Hedgehog signaling, such as glioma-associated oncogene homolog (Gli)-1, Gli-2, Gli-3, Indian hedgehog homolog (Ihh) and Fox-F1 in the development of intestinal atresias has been excluded by recent studies (178,179). Last but not least, mutations of Cdx-2, which is known to be expressed only in the hindgut, elicit colonic atresia as well (70). It is a fact that atresias begin to emerge during the stage of rapid intestinal elongation when the developmental rate of intestine greatly outpaces the developmental rate of the whole embryo.

Recanalization disorders: Duplication. Another rare, yet possible malformation during colonic embryonal development is colonic duplication. The extra lumen may be spherical or tubular and it may or may not communicate with the major lumen (180). However, to date, no clear etiology for this condition has been recognized. Among various hypotheses, one refers to persistent intestinal outpouchings after their creation during week 6 to 8 of gestation. Moreover, another possible cause is attachment between gut endoderm and neural tube ectoderm, which pulls gut towards the vertebra and does not allow it to separate from the ectoderm. Developmental traction later leads to the creation of tubular outpouchings, which produce the duplication. Another theory suggests that insufficient recanalization following the obliteration of the GI tract at week 6 of gestation eventually creates two lumens instead of one (181). Finally, some other theory involves vascular events in the pathogenesis of duplication (182).

Morphogenesis of the epithelium and adjacent mesenchyme. Following the recanalization of the GI lumen, the endoderm begins to mature in a site-specific manner. The 9th week of gestation is the time when the initial cuboidal stratified epithelium of midgut and hindgut begins to alter into the future simple columnar epithelium. In the meantime, the mesenchyme and outer mesothelium surrounds the epithelium (183). During week 10, villus-like structures emerge and longitudinal shafts form secondary lumens (184,185). Simultaneously, mesenchyme invaginates into the epithelial shafts and forms longitudinal folds, which convert to villi with stratified epithelium. The epithelial cells differentiate and goblet cells are visible for the first time during 11-12th week of gestation (186). Subsequently, epithelial and mesenchymal rearrangements lead to dissolution of the primary villi. Crypts emerge as crypt-shaped formations of the secondary lumens in the basal layers of the epithelium. In the meantime, mesenchymal cells invade between adjacent epithelial layers and divide primary villi into smaller villi, consisting of mesenchymal lamina propria and an overlying simple columnar epithelium (185). Notably, the initial embryonal structure of large intestine not only includes crypts, but also villi (87,184).

The presence of villi polarizes the epithelium and the underlying mesoderm creating a basal side, with crypts inside the submucosa, and a luminal villus, which contains epithelium that changes pertaining to the differentiation stage and the proliferation rate along the crypt-villus axis. Generally, crypts contain less well-differentiated cells with higher proliferation rate, whereas in the villus tip cells are terminally differentiated, specialized, with minimum proliferation rate. Epithelial reorganization, which constantly occurs during development, requires this apico-basal polarity, which is controlled by various signaling pathways (108). There is sufficient evidence to indicate that Wnt5a is one of the regulators of the intestinal epithelial architecture, as it mediates part of non-canonical Wnt signaling. Wnt5a null mice exhibit the deregulation of the apical-basal polarity of the intestinal epithelium, as post-mitotic cells remain in apical layers and do not attach to the basal membrane, resulting in the hindering of gut elongation (107). Concurrently, the insufficient functioning of the sFRP inhibition over Wnt5a also lead to deregulation of the intestinal epithelial apical-basal polarity. To elaborate, the inactivation of sFRP provokes the formation of epithelial clumps due to defective intracellular organization of the epithelial cells rather than epithelial overproliferation (108). Therefore, Wnt5a balanced activity is important for the proper development over both the AP and RAD axis. Ezrin also seems to be a key factor in Wnt/PCP signaling and its absence leads to improper polarization with villus fusion and mucosal disorganization, even though no disturbance is apparent early during gut development (187).

Epithelial-mesenchymal interaction is of major significance during the patterning of the epithelium along the RAD axis. Various experiments have been conducted to evaluate the effects of the endoderm over the mesoderm and vice versa

when they originate from different tissues. When the proximal jejunum endoderm is co-cultured with proximal colon mesenchyme, it forms villi, which contain jejunum specific sucrase-isomaltase expressing enterocytes and endocrine cells that express the jejunum specific cholecystokinin (CCK) hormone. However, small intestine-specific Paneth cells are not apparent in this epithelium. If proximal colon endoderm is mixed with proximal jejunum mesenchyme, villi form again and they contain sucrase-isomaltase enterocytes as well. However, Paneth cells are apparent, whereas the endocrine cells express distal type hormones peptide-YY (PYY) and glucagon-like peptide (GLP)-1 instead of CCK (188).

The mesenchyme expresses and secretes various signaling molecules which significantly affect endodermal differentiation. If the secretion of vesicles through the the plasma membrane of mesenchymal cells is deregulated, for example by a mutation in epimorphin, epithelial morphogenesis becomes is and epithelial proliferation is accelerated (189,190). Epimorphin is a protein which is implicated in the vesicle secretory system of mesenchymal cells (191) and its inhibition probably disrupts endodermal-mesenchymal intercellular signaling pathways, such as BMP and Hedgehog. Plenty of other signaling molecules are implicated in the intercellular coordination, such as Wnt, FGF, epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and TGF-β.

The maturation of the mesenchyme is largely regulated by signals of the overlying endoderm. Hedgehog molecules are among the most important mediators of these signals. During the early development of the pseudostratified epithelium, Shh and Ihh are expressed by the intestinal endoderm. As long as development proceeds, Ihh continues to be traced throughout the whole endoderm, whereas Shh expression gradually becomes limited to the villus base, being restricted to the less differentiated progenitor cells and eventually stops (192). In Shh mutations, villi overgrow, whereas in Ihh mutations, epithelial proliferation decreases and the villi become fewer and smaller. Therefore, Shh and Ihh may provoke opposite results (178). Total Hedgehog inhibition via various mechanisms leads to the defective development of the intestinal mesenchyme with immature epithelium and the disruption of the organization of villi. Moderate inhibition of Hedgehog disorganizes (193,194) the differentiation of the epithelium along the RAD axis, as crypts ectopically form and branch inside villi (193,195).

The transcription factors, Gli-2 and Gli-3, have been found to act as subepithelial mesenchymal mediators of Hedgehog signaling by activating transcription factors of the Forkhead box winged-helix superfamily (196). Mutations to Fox-L1 or forkhead homologue (Fkh)-6, which are genetic targets of Gli, lead to the developmental reorganization of the epithelium and villi, with concurrent hyperproliferation and deregulation of crypts and their branching (197). Fox-F1 and Fox-F2 are also targets of Hedgehog signaling and participate in mesenchymal differentiation, elongation and maintenance. The silencing of these factors provokes the disintegration of the mesenchyme shortly after the formation of villi (198). Gli and Fox molecules cooperate with other signaling pathways, such as Wnt and BMP, which hormonally adapt endodermal development in coordination with mesenchyme.

BMPs, particularly BMP-2, 4 and 7 are expressed in the subepithelial mesenchyme, mostly under nascent villi and have been reported as downstream targets of Hedgehog (49). In the chick hindgut, the derangement of BMP signaling results in impaired development and the differentiation of all three layers of gastrulation (199). Mutation of the BMP receptor (BMPR)1 facilitates proliferation, crypt development in villi and polyp formation, leading to juvenile polyposis (200,201). If BMPR1 is inhibited only in the epithelium, proliferation still increases, although no polyps form (202). The epithelial inhibition of BMP by the antagonist, Noggin, leads to the less compact development of subepithelial mesenchyme with an unaffected epithelium. As a consequence, larger, but fewer villi form, whereas, in later developmental stages, crypts ectopically proliferate in the villi and polyps form in the intestines. Probably, Wnt, PDGF, Hedgehog and other signaling pathways contribute to this pathology as well (193,203). It is evident that BMP is crucial for the configuration of the RAD intestinal axis.

PDGF signals from the epithelium towards the mesenchyme, such as Hedgehog. PDGF-A is expressed in the endoderm even before the formation of villi and gradually becomes restricted in the lower parts of the villi and the crypts. Its receptor, PDGFR-α, is expressed in the mesenchyme simultaneously with PDGF-A, while its expression is enhanced beneath nascent villi, at the growing spot of the elongating villi. The genetic inhibition of PDGF signaling disrupts the mucosal architecture in colon, possibly due to the early differentiation of mesenchymal smooth muscle, in spite the fact that proliferation continues to occur in crypts and in the intervillus epithelium (194).

EGF and its receptor, EGFR, are also implicated in intestinal development. The deletion of EGFR delays villus emergence, with consequent diminished proliferation, villus blunting and disintegration of tissue. However, these observations vary among different species and different developmental stages of intervention.

E74-like factor (Elf)-3 cooperates with CR6-interacting factor (Crif)-1, which is a transcriptional co-activator, to facilitate the emergence of villi. Mice with either Elf-3 or Crif-1 deficiency exhibit a diminished expression of TGF-β receptor (TGF-βR)II, with concurrent fewer, malformed villi, disorganized lamina propria and malfunctioning epithelial cells. The re-expression of TGF-βRII has been shown to rescue the normal phenotype with proper epithelial differentiation, and it has been suggested that Elf-3/Crif-1 co-mediate villus emergence via a procedure that is mediated by TGF-β signaling (204-206).

Mutations of the mesenchymal factor, NK2 homeobox 3 (Nkx2.3), lead to mesenchymal cell reduction, lower epithelial proliferation, the delayed emergence of villi and a high risk of intrauterine death. If mice manage to survive, they exhibit a rebound epithelial hyperproliferation and mucosal thickening with branched villi and abnormal architecture (207).

Intestinal development also requires chromatin remodeling. Mutation of the p300 histone acetyl-transferase (HAT) delays villus emergence, with failure of subepithelial mesenchymal condensation and decreased BMP-4 expression at the points of perspective villi. However, a similar mutation of the HAT protein CREB-binding protein (CBP) does not alter intestinal embryological development (208).

The opposite procedure of HATs is conducted by histone deacetylases (HDACs). HDAC-1 and 2 are highly expressed in the early intestinal endoderm, whereas they become confined to the villi following the emergence of the villus. The overexpression of HDACs has been found to block epithelial differentiation, while HDAC inhibition leads to the increased histone acetylation of epithelial cells and the subsequent immaturity of villus development and epithelial differentiation (209).

Epithelial cytodifferentiation. Cytodifferentiation along the RAD axis is based on interaction with the underlying mesoderm, interaction with basal membrane proteins and contact with luminal nutrients in some species. There is no evidence that functional cytodifferentiation begins before villus emergence, when proliferation is very rapid, even though at that time there is some functional barrier to the passive diffusion of macromolecules (210). During the initial stages of endodermal differentiation in the colon, there is a simultaneous conversion of the pseudostratified epithelium towards a simple columnar epithelium, the emergence of villi and the augmentation of the epithelial proliferation rate at the villus bases. The centers of proliferation are later altered, traced initially in the intervillus epithelium and finally inside the crypts of Lieberkühn.

Four main epithelial cell types are distinct during villus emergence in the large intestine using molecular and functional markers: i) The main absorptive cells are columnar enterocytes, while the class of secretory cells includes; ii) mucous-producing goblet cells; iii) caveolated or tuft cells; and iv) various hormone-producing enteroendocrine cells. It is not clear whether there are specific stem cells which differentiate to each one of these cellular categories. Two of the major molecular pathways which are implicated in the modulation of proliferation and cytodifferentiation are Wnt/β-catenin and Notch.

As it has already been stated, Wnt/β-catenin mediates part of the stem cell maintenance, proliferation and cytodifferentiation. Several factors of the Wnt/β-catenin pathway are traceable in the developing endoderm and mesoderm. It seems noteworthy that β-catenin is transcriptionally active after villus emergence, being detectable restrictedly in the post-mitotic cells before birth (90,211). After birth, β-catenin redistributes and is expressed in the intervillus epithelium. However, there is much controversy about this evidence.

Wnt/β-catenin signaling and its final purpose can be disrupted via various mechanisms. In the adult intestine, if Wnt/β-catenin is directly inhibited, the acute loss of proliferation occurs, with the depletion of progenitor cells and the concurrent arrest of the cytodifferentiation of secretory cells (212,213). Therefore, the canonical Wnt pathway is necessary at least for the maintenance of the proliferative potential of the intestinal epithelium. In the embryonic intestine, Tcf-4 is expressed in the intervillus epithelium, whereas Tcf-3 is expressed in the villus epithelium. Taking into consideration that β-catenin is traceable only in the embryonic mature villus epithelium and Tcf-4 is restricted to the intervillus epithelium, it is evident that β-catenin probably utilizes different Tcf mediators, such as Tcf-3, or totally different families of signaling factors, such as Sox, to achieve its purpose (211,214).

However, if β-catenin is primarily or secondarily prematurely activated in the intestinal endoderm, villi do not emerge normally and cytodifferentiation is disrupted, even though this may be a consequence of the fact that the endodermal differentiation radically alters towards non-intestinal tissues as endoderm no longer expresses Cdx-2 (90,215).

The Notch signaling pathway consists of transmembrane receptors, which bind several adjacently secreted ligands and respond by releasing a cytoplasmic transcriptional activation domain, the Notch intracellular domain (NICD). This domain travels intracellularly, enters the nucleus and binds to CBF-1, suppressor of hairless, Lag-1 (CSL)/recombination signal-binding protein-J (RBP-J) proteins facilitating the transcription of target genes. Hairy and enhancer of split (Hes)-1 is one of these genes and its activation alters the equilibrium of the cytodifferentiation, favoring the formation of enterocytes against secretory cells (216). The activity of Hes1 represses atonal homolog (Atoh)-1/Math-1, which are significant for the manifestation of the secretory phenotype (217-219). Thus, Notch signaling, orients epithelial cytodifferentiation towards absorptive or secretory cells just by adjusting the balance between Hes-1 and Atoh-1 expression.

Notch signaling does not act independently, but it crosstalks with Wnt/β-catenin signaling (212). Usually, it seems that Notch antagonize Wnt signaling trying to facilitate absorptive cytodifferentiation. Notch signaling enhances the GSK-3β-mediated degradation of β-catenin (220). Concurrently, Notch blockade augments Wnt signaling with subsequent differentiation towards the secretory phenotype. When Wnt inhibition and Notch inhibition coexist, secretory cytodifferentiation normalizes (221). Of note, the intestinal inhibition of Wnt suppresses epithelial proliferation and its manifestations resemble Atoh-1 mutation as secretory cytodifferentiation is repressed (217). When the Wnt/β-catenin pathway functions properly, it enhances the effects of Notch signaling by upregulating its receptors and the expression of its downstream ligands, and by activating the targets of Notch signaling by itself. One of these targets is Hes-1 (222). Simultaneously, in the presence of usual Wnt activity, the APC/axin complex degrades Atoh-1 altering the equilibrium towards an absorptive phenotype, whereas β-catenin has been shown to have Atoh-1 as a transcriptional target, reinforcing its activity (223). Various factors can affect both Notch and Wnt signaling, such as the reactive oxygen species (ROS)-producing NADPH oxidase (Nox)-1 (224).

Crypt development. Crypt formation begins with the transposition of stem cells in the intervillus epithelium and synchronized tissue remodeling, resulting in the final form of the crypts. Crypts extend by the translocation of the crypt-villus junction upwards (225). *In utero*, stem cells in crypts are polyclonal, whereas the postnatal stem cell population becomes monoclonal (226). Polyclonality possibly derives from two major facts, the rapid epithelial proliferation of the intestine and the fission-way of crypt expansion. Rapid proliferation induces each stem cell to create its own perimeter of daughter cells, resulting in patches with monoclonality in the center and polyclonality in the periphery of each patch. In the meantime, the fission of existing crypts forms new crypts contributing to the increase in polyclonality. After birth, the rapid proliferation of resident stem cells gradually restores the initial monoclonality.

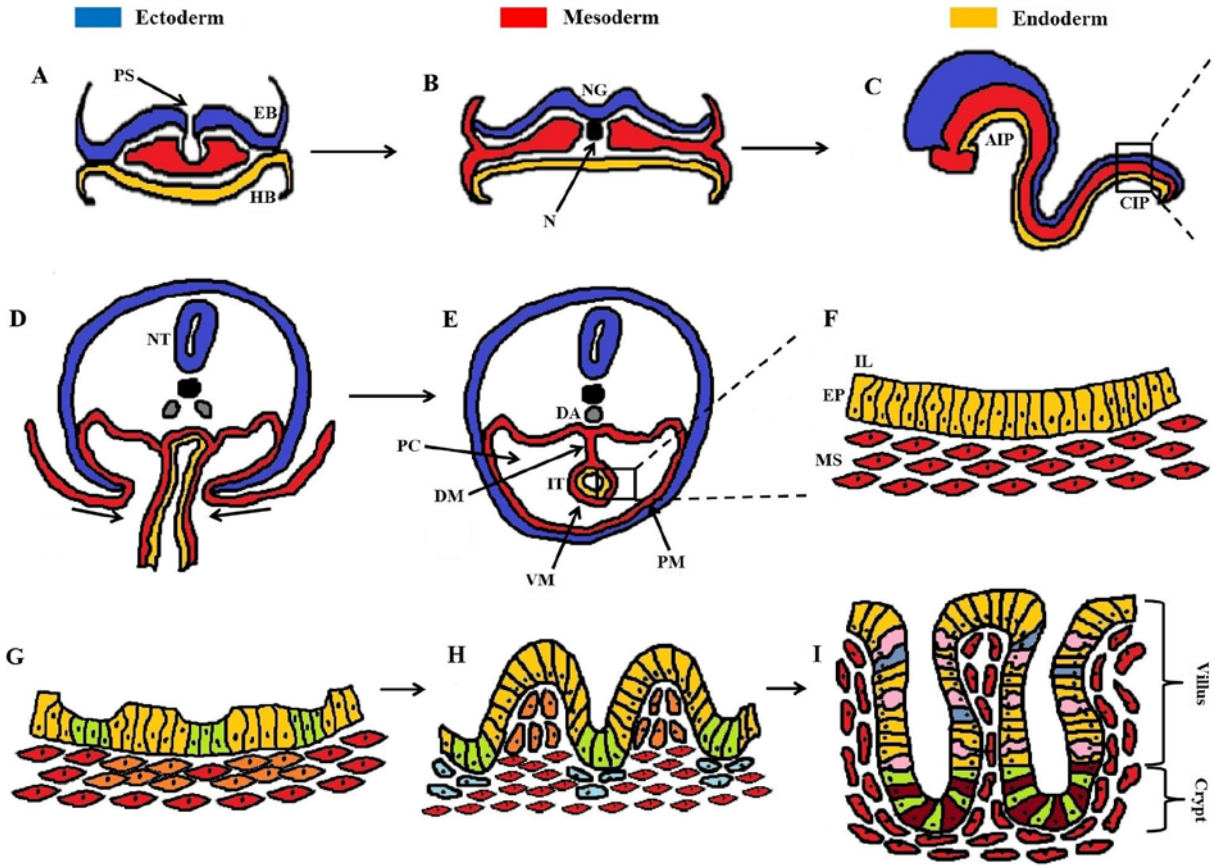

Figure 1. Developmental stages of the intestinal epithelium. (A) Gastrulation with migration of cells from the primitive streak, performing either EMT and/or MET. (B) Formation of the three germinal layers (ectoderm, mesoderm, endoderm). (C) The primitive gut tube is formed through invaginations in the AIP and CIP, consisting of the endoderm (internally) underlying the mesoderm (externally). (D) Cross-section of the rectangular area in (C), showing the ventral invaginations of mesoderm. (E) Formation of the peritoneal cavity around the closed IT, with PM and VM. (F) Magnification of the rectangular area in (E), showing the pseudostratified structure of the intestinal epithelium (EP, yellow cells) and underlying mesenchyme (MS, red cells). (G) Formation of mesenchymal clusters (orange cells) as a response to epithelial signaling, marking the onset of villus morphogenesis. (H) Progressive epithelial remodeling via mesenchymal signaling polarizes the columnar epithelial cells into shaping stereotypical villi (yellow cells) and proliferative intervilli (green cells). Mesenchymal clusters at the top of villi prevent further epithelial proliferation through post-mitotic signaling, whereas clusters below the intervilli (blue cells) regulate the division of intestinal stem cells. (I) Maturation of intestinal epithelium with definite formation of villi and crypts housing mostly enterocytes (yellow cells), goblet cells (pink cells), and enteroendocrine cells (blue cells). At the base of the crypt the cellular populations are mainly dominated by secretory Paneth cells (dark red cells) and proliferative intestinal stem cells (green cells). A dense network of myofibroblasts (red cells) underlies the intestinal epithelium. AIP, anterior intestinal portal; CIP, caudal intestinal portal; DA, dorsal aorta; DM, dorsal mesentery; EB, epiblast; EMT, epithelial-mesenchymal transition; EP, epithelium; HB, hypoblast; IL, intestinal lumen; IT, intestinal tube; MET, mesenchymal-epithelial transition; MS, mesenchyme; N, notochord; NG, neural groove; NT, neural tube; PC, peritoneal cavity; PM, parietal mesoderm; PS, primitive streak; VM, visceral mesoderm.

The main molecular pathways which are implicated in the development of crypts are BMP and Hedgehog. BMP signals restrict stem cells in the emerging crypts, partly via the inhibition of the Wnt/β-catenin pathway through the phosphatase and tensin homolog (PTEN)/phosphoinositide 3-kinase (PI3K)/AKT pathway (227). Wnt/β-catenin mediates the expression of ephrin type-B (EphB)2 and EphB3 in the intervillus epithelium and the restriction of the EphrinB1 in the villus epithelial cells. Eph/EphB function modulates the formation of crypts by allowing the maintenance of proliferation initially in the intervillus epithelium and later inside the crypts (228). Moreover, β-catenin-targeted genes can be used as markers of the crypt-base stem cells. For instance, the expression of achaete-scute complex homolog (Ascl)2, which is a β-catenin target, augments proliferation and boosts crypt formation, whereas its deletion leads to the inhibition of stem cell replication and subsequent vanish of crypts (229). Nevertheless, further research is required in order to elucidate the mechanisms of the morphogenesis of the colonic crypts. The developmental stages of the intestinal epithelium are depicted in Fig. 1.

9. Enteric nervous system (ENS)

The development of the ENS is a necessary condition for the functionality of the mature colon. In the ENS, >100 million sensory, motor neural cells and interneurons exist, being supported by glial cells, both of which are products of the same progenitor cells (230,231).

The impairment of ENS embryonal development can result in possible developmental disorders, such as colonic dysmotility (232). The most usual congenital clinical syndrome owing to abnormalities during the development of the ENS is Hirschsprung disease (HSCR), in which the ENS fails to establish its position along the GI tract, resulting in functional intestinal obstruction of various lengths. It usually

involves only the distal colon, although there are cases of the total absence of the ENS across the entire GI tract (233,234). In this condition, the pathological intestinal section is contracted, empty of content and unable to participate in a peristaltic wave.

Developmental principles of the ENS. Firstly, the compartment of the progenitor cells of the ENS derive from neural crest and these specific cells are called enteric neural crest cells (ENCCs). These ectodermal cells, delaminate from the gradually closing cranial neural folds, migrate through the mesenchyme and endoderm, colonize the GI tract and differentiate towards the different cell types of the ENS (235,236) If the dorsal neural tube of chicks is removed, they fail to develop an ENS (237). The major initial source of ENCCs is the vagal neural crest, from neural tube at the levels of somites 1-7 (236,238). The sacral neural crest also contributes to the ENS, by providing progenitor cells for the development of enteric neurons and glial cells caudally to the umbilicus (239). Different sections of the GI tube receive ENCCs form different axial levels of the neural tube (238). The somite levels 1-2 neural crest contributes only in esophagus, ENCCs from somite levels 3-5 colonize regions from stomach to hindgut and somite levels 6-7 provide progenitor cells only to the hindgut (238,240). Therefore, it is evident that the ENS of the colon is created by cells from neural crest cells (NCCs) below somite level 3.

As far as the ENS is concerned, at somite stage 13, a population of NCCs originating from somites 1-3 migrate ventrally and gradually form the sympathetic and dorsal root ganglia, while a proportion of them colonizes either the heart or foregut to begin the formation of ENS (238,241). NCCs originating from the rest of the vagal neural crest, at somites levels 4-7, migrate only ventrally and reinforce the colonization of GI tube. In contrast to vagal-derived NCCs (242), the more caudal truncal NCCs are not able to enter the foregut, as they express Robo, which interacts with Slit-2 that is expressed in the foregut, resulting in rejection from the foregut (243). After exiting the neural crest, vagal NCCs cross the mesoderm between the sclerotome and dermomyotome. Before they enter the intestinal mesenchyme, they form ganglia next to it (244). It seems that during this passage, paraxial mesoderm produces RA, which binds to RA receptors of NCCs, activating the expression of receptor tyrosine kinase rearranged during transfection (RET) by NCCS. This step probably represents the future restriction of these NCCs as progenitors only for ENS. The deficiency of the necessary RA signaling mediator Raldh-2 leads to ENS agenesis, linked to the decreased expression of RET.

After the entering of vagally-derived ENCCs into the foregut, they migrate caudally as a waveform process with a speed of 40 μm/h (245). Their migration occurs randomly inside the outer mesenchyme, where smooth muscle has not yet emerged (246,247). In the midgut, while the caudal extension of ENS continues, smooth muscle begins to form and ENCCs become limited between the circular and the longitudinal muscle layers, forming the myenteric plexus. A secondary migration wave guides ENCCs from the myenteric plexus towards the submucosal region, establishing the submucosal plexus (247). It is noteworthy that the two plexuses develop early and simultaneously in human and avian organisms (248). In humans, the total AP colonization of the GI tract by ENCCs is completed by week 7 of gestation (248).

Apart from the vagal neural crest, the sacral neural crest, which is located caudally to sacral level 28, also contributes to ENS of the GI tract, particularly in the colon and rectum. To elaborate, sacral NCCs also travel ventrally, migrate through somites and mesenchyme, and reach regions laterally of the cloaca and caudal hindgut in order to give rise to the pelvic plexus. Following the arrival of NCCs from the vagal neural crest in the hindgut, cells from the pelvic plexus set out to colonize the distal hindgut and provide additional neurons and glial cells to the ENS (240,249).

Compared to sacral NCCs, vagal NCCs are far more invasive, partly being justified by the fact that they express four-fold higher levels of RET (250). If RET is upregulated in sacral NCCs, their invasiveness and potential for migration increases, whereas if the sacral neural tube gets replaced by vagal neural tube, these NCCs also reach distal colon much earlier (251). Alternative paths for the neural colonization of the gut and particularly colon have been presented. It has recently been proven that Schwann cell progenitors in extrinsic nerve fibers also contribute 20% of the hindgut ENS (252). Moreover, the waveform process may not be totally continuous along the AP axis of the intestine. Specifically, some of the caudally migrating ENCCs skip cecal mesenchyme, cross the midgut mesentery and reenter gut more distally. This phenomenon is so profound that >50% of the ENCCs of distal hindgut follow this method of migration (253).

The key features of the developing ENS, which have to be carefully regulated, are cell proliferation, cell survival, migration and its direction, the creation of concentric plexuses, cytodifferentiation and the formation of ganglia, axis and synapsis.

One the most important factors during ENS formation is the quantity of ENCCs, since the reduction of the size of vagal neural crest leads to distal aganglionosis (254). Moreover, the extension of ENCCs occurs in a chain pattern and is facilitated by close cellular contact, whereas individual ENCCs migrate at a much lower speed (255,256). Proliferation increases the cellular density and augments contact of cells. It seems that the cellular contact is perceived by cell to cell adhesion and requires adhesion molecules, such as L1 cell adhesion molecule (L1CAM) (257). It is noteworthy that, when ENCC density is relatively low, the decreased speed of their migration is not sufficient, as the mesenchyme matures and alters its conditions, which are no longer favorable for the arrival of ENCCs (258).

Another principal is that, across the AP axis of migration, cells in the wavefront have to maintain their proliferative potential and migrate distally, whereas cells behind the wavefront need to slower their proliferation rate and start to maturate. The migration progresses from regions of high cellular density and low availability of neurotrophic factors, such as glial-derived neurotrophic factor (GDNF) due to depletion, towards regions of higher ENCC carrying capacity, which happen to exist distally (251,259). Another mechanism that may regulate this migration stream and its termination could be the existence of some negative signal among ENCCs when there is no neighboring tissue, without ENCCs for them to head, and they inevitably contact each other (260).

Once the wavefront reaches each compartment of the GI tube, including the colon, ENCCs have to stop proliferating and begin differentiating. If proliferation occurs at a relatively slow rate, there is no sufficient number of ENCCs to colonize the whole gut, with the colon being the most affected part as the last compartment which becomes colonized. The premature differentiation of otherwise proliferative ENCCs also decreases the migratory potential of the wavefront, leading to a variable extent of aganglionosis. On the contrary, differentiation rates which are too slow result in abnormal neural and glial maturation as the mesenchyme, which matures more rapidly, no longer facilitates cytodifferentiation at the late time when that begins (261).

The innervation of colon has been found to be embryologically configured by several concurrent and complex mechanisms and provide a broad area of research.

Molecular signaling in enteric innervation. RET is a tyrosine kinase transmembrane receptor, expressed by ENCCs and mediating migration (262,263). Both Sox-10 and paired-like homeobox (PHOX)-2B are necessary for the expression of RET, or total aganglionosis otherwise occurs (264,265). One of the RET activators is GDNF, which is expressed in the mesenchyme. GDNF binds to the complex RET-GDNF family receptor (GFR)a1, provoking the RET phosphorylation and activation of multiple intracellular pathways, including Ras/mitogen activated protein kinase (MAPK), JNK and PI3K (266). PI3K activation is of upmost significance as it mediates the GDNF-guided proliferation, migration and survival (267,268). PI3K normally mediates the conversion of phosphatidylinositol (3-5)-trisphosphate (PIP3) to phosphatidylinositol 4,5-bisphosphate (PIP2), whereas its inhibition leads to distal aganglionosis due to limited migration (269,270). PTEN antagonizes PI3K, catalyzing the opposite reaction, the conversion of PIP2 to PIP3. As is evident, PTEN is not normally expressed in the tip of the wavefront as its expression would compromise the migration potential. RA signaling concurrently induces RET expression in NCCs, patenting them as ENCCs, and accomplishes PTEN absence, maintaining the effectiveness of PI3K in facilitating proliferation and migration (270,271). On the other side, PTEN is present and is very useful in regions proximal to the wavefront, where it is needed to terminate proliferation and migration (270). Additional molecular factors, which try to balance the function of RET-GDNF interaction are Sprouty homolog (SPRY)-2 and KIF26A. Enteric nerve hyperplasia occurs if either of their genes is silenced (272,273).

Apart from proliferation, RET also maintains the survival of ENCCs, which is necessary for migration (274,275). The intestinal length where aganglionosis occurs is proportional to the degree of RET insufficiency. $RET^{+/-}$ mice exhibit an almost normal intestine with proper innervation, the reduction of RET expression to one-third of its normal value, provoking moderate colorectal aganglionosis, whereas $RET^{-/-}$ mice present with total aganglionosis (276,277). GDNF possibly emits some chemoattractive signals, which direct migration (278), whereas the absence of GFRa1 attenutes ENCC migration (279). It is noteworthy that the RET-GDNF interaction contributes to the migration of ENCCs towards the submucosa layer as well (279).

There are indications that RET-GDNF somehow regulates cytodifferentiation, even though the research results are controversial (275,279,280).

Endothelin receptor (EDNR)B is a G-protein receptor expressed by ENCCs, whereas its ligand, endothelin (ET)-3 is expressed in the mesenchyme. EDNRB signaling is required in regions caudal to the cecum (255) and it serves to facilitate proliferation and inhibit cytodifferentiation (274,281). The deletion of the genes of EDNRB or ET-3 or of the endothelin converting enzyme (ECE)-1 gives rise to colorectal aganglionosis (282). The loss of EDNRB signaling suppresses proliferation and promotes cytodifferentiation. There is evidence of the EDNRB interaction with RET signaling, since either RET heterozygous mutation or EDNRB homozygous mutation do not raise any severe abnormality, whereas their combination provokes aganglionosis (283,284). Furthermore, ET-3 reinforces GDNF-mediated proliferation (274), even though it antagonizes GDNF-mediated ENCCs cytodifferentiation towards neural cells (285).

Sox-10, an HMG box-containing transcriptional factor, expressed by NCC and later by ENCCs, is required for the survival of ENCCs, along with the maintenance of the proliferative potential and the inhibition of cytodifferentiation (286). Heterozygous Sox-10 mutation decreases the ENCC population and promotes premature differentiation to neurons and distal aganglionosis (287), whereas homozygous Sox-10 mutation provokes total aganglionosis due to the failure of NCCs to survive until they reach the foregut (264). Sox-10 may succeed its purpose via the direct activation of RET and EDNRB (288,289). Following the cytodifferentiation of ENCCs, Sox-10 expression is terminated in neurons, whereas it continues in glial cells (261).

PHOX-2B, a transcriptional factor expressed in ENCCs following their invasion inside the mesenchyme, promotes proliferation and survival and mediates the formation of the enteric ganglia (290). Its deletion results in total intestinal aganglionosis (265). There is antagonism between Sox-10 and PHOX-2B inside cells, as they suppress each other. ENCCs differentiating towards neurons express PHOX-2B, in contrast with glial cells, which express Sox-10 (291). The msutation of PHOX-2B leads to Sox-10 dominant expression and differentiation towards glial cells, not neurons (292).

Heart- and neural crest derivative (HAND)-2, also a base helix-loop-helix transcription factor expressed in ENCCs following their arrival in the foregut, mediates differentiation to mature neural cells and the specification of neuro-transmitters (293). When HAND-2 expression is expressed, intestinal motility becomes impaired (294), whereas HAND-2 deletion leads to lower numbers of enteric neurons and disrupted ENCC differentiation into neurons (293,294).

BMP-2 and BMP-4 affect migration and regulate the balance between neural versus the glial differentiation of ENCCs (295-297). They also facilitate the formation of enteric ganglia and a lower neural density. If BMP signaling is inhibited, for instance due to Noggin, the neural-to-glial ratio increases (297). Moreover, BMPs promote the domination of neural cells which express tropomyosin receptor kinase (Trk)C, which is a tyrosine kinase receptor that binds ET-3 and reinforces survival and differentiation (296). The deletion of either TrkC or ET-3 diminishes the neural cell pool (298).

Role of extracellular matrix in ENS development. Apart from the receptors and transcription factors, which affect the key features of the development of the ENS, another necessary condition for the proper distribution and differentiation of ENCCs is a suitable microenvironment. This is constructed by components of the ECM. When ET-3 is ablated, laminin, perlecan and collagen type VI are traced in increased concentrations, supporting the statement for the crucial contribution of the ECM in the patterning of ENS (299,300). ENCCs have receptors in their cellular surface, while ECM can alter survival, proliferation, migration, differentiation, gangliogenesis and the formation of the two plexuses.

Migration has been proven to be facilitated by the presence of laminin, fibronectin, vitronectin and collagen type I, whereas it is inhibited by collagen type VI and possibly by chondroitin sulfate proteoglycans, such as versican (301-305). The ECM significantly contributes to the RAD patterning of ENS, creating mesenchymal boundaries which are not permissive for the development and extension of ENCCs. As a consequence, ENCCs are forced to position themselves along two permissive regions where the two enteric plexuses form (305). Moreover, it is noteworthy that while ENS formation proceeds, ECM in the midgut and hindgut expresses greater concentrations of collagen type I and gradually becomes stiffer, decreasing the potential speed of ENCC migration (306).

However, it seems that ENCCs produce their own ECM proteins, which they secrete in their surrounding ECM in order to ameliorate their microenvironment and augment their migratory potential. This is particularly observed in vagal NCCs, not in sacral NCCs, partly justifying their different migratory capability (301). ENCCs have also been found to secrete metalloproteinases (MMPs) in the ECM to degrade part of it and make it less dense and stiff in order to ease their passage, according to their direction. When MMP-2 is inhibited, the migratory potential of ENCCs is reduced (307). Furthermore, in the case of the upregulation of collagen-6a4 gene, the secreted collagen VI in ECM is increased and ENCC migration is confined (304).

ENCCs interact with the ECM via their surface adhesion molecules, which bind to ECM molecules, such as L1CAM, N-cadherin and numerous integrins (302,303,308). L1CAM inhibition disrupts the close contact of ENCCs in the wavefront tip, which is required for migration to proceed (309), while the deficiency of either L1CAM or N-cadherin attenuates ENCC migration (257,310). As far as laminin is concerned, even though it initially is non-specifically expressed in mesenchyme, it later becomes restricted at the basal lamina of epithelia, endothelial cells and smooth muscle cells. The pertinent endothelial cells form two concentric capillary plexuses in mesenchyme, the endothelial cells of which maintain laminin expression. The migratory ENCC wave colonizes the mesenchyme in close contact with these endothelial cells, suggesting that the two concentric capillary plexuses act as definers of the future position of the two concentric ENS plexuses, in a process which also requires integrin-β1 (302). The disruption of the formation of capillary plexuses provokes distal aganglionosis which implicates the colon (302).

Epithelial signals regulate ENS formation. The epithelium, either with direct signals or with indirect signals via the mesenchyme, regulates ENS formation vai signaling that implicates Netrin and hedgehog (305). Epithelial cells produce netrins, which are extracellularly secreted and direct axon formation, acting in a chemoattractive manner on neural cells that express deleted in colorectal cancer (DCC). Netrins have also been proven to guide ENCC migration across the RAD axis, from the myenteric to the submucosal plexus. It is noteworthy that DCC mutant mice do not develop the submucosal neural plexus, even though they exhibit a normal Netrin expression (311).

On the other side, endodermally-derived Hedgehog signaling, which includes Shh and Ihh, can alter mesenchymal proliferation and differentiation, which can alter the microenvironment where the ENS develops. Shh also contributes to the concentric pattern of the ENS (2). Hedgehog signals reach the mesenchyme, which responds by changing the degree of BMP-4 expression. BMP-4 inhibits mesenchymal differentiation towards smooth muscle, potentiating ENCC migration, particularly towards the submucosal layer (49,52,295). Moreover, Hedgehog induces the mesenchymal expression of Fox-F1 and Fox-F2, the inactivation of which diminishes the presence of collagen type-I and IV in ECM. As a result, the ECM is less conducive for the development of ENS and colorectal aganglionosis occurs (198).

Shh signaling over the mesenchyme is partly mediated by the mesenchymal receptor patched-1 (PTC1) and the downstream smoothened-Gli cascade. Both Shh inhibition and deficiency provoke the emergence of ectopic ganglia in the submucosal layer with increased neurons and malformed neural projections (178). On the contrary, Shh overexpression provokes intestinal aganglionosis, supporting some inhibitory action of Shh over the development of the ENS (305). Endorsing this statement, Shh overexpression has also been found to increase the mesenchymal expression of chondroitin sulfate proteoglycans even ectopically, resulting in the hampering of ENCC migration and consequent intestinal aganglionosis (305). The role of Ihh in ENS development has not been adequately investigated.

It is noteworthy that, even though the significance of Shh in ENS development seems undoubtable, its effects on ENCCs probably occur indirectly by other mediating tissues. To elaborate, no receptors for Shh have been traced in ENCCs, whereas PTC1 is expressed only by the intestinal mesenchyme (305).

HSCR. As it has already been reported, HSCR is a congenital condition which usually involves the distal GI tract, most frequently sigmoid and rectum, and is also referred as congenital megacolon or intestinal aganglionosis. It has an incidence of 1:5,000 and a male predominance of 4:1 (312). In HSCR, the ENS does not succeed to develop in the respective intestinal segment resulting in inability of propelling the intestinal content out of the GI tract. The aganglionic segment is collapsed, without content, whereas the more proximal intestinal parts distend, contributing to the phenotype of megacolon. It is noteworthy that some intestinal length proximal to the totally aganglionic compartment also exhibits some developmental hypoganglionosis (233).

HSCR is one of the few congenital conditions which has attracted extensive research into its underlying defects. RET mutations are the most frequent of the known mutations that

have been traced in HSCR. However, in >50% of cases, none of the previously noted mutations are apparent. A number of different genes and signaling pathways have been implicated in its pathophysiology (262,263).

A particular RET mutation has been found to delay the transmesenteric passage of ENCCs on their way to reach the hindgut, which later makes it difficult for ENCCs to cross the already maturated mesenchyme and increases the risk of developing agangliogenesis of the distal colon (253). Mutations of non-coding regions in RET have also been proven to increase the risk of HSCR, which probably still occurs owing to the existence of other mutations (263,313,314).

Another possible reason for HSCR is reduction of the progenitor pool. This can occur either due to GDNF insufficiency, which confines ENCC proliferation and migration (315), or owing to irregular interaction among Sox-10, ET-3 and EDNRB, which normally maintains ENCCs in an undifferentiated, proliferative state (274,316,317). In particular, ET-3-EDNRB signaling has been proven to be more important for the development of ENS in distal colon, during the latest phases of intestinal colonization (318). If heterozygous Sox-10 mutant mice also experience some loss of EDNRB, they exhibit a phenotype similar to that of HSCR, without any apparent increase in ENCC apoptosis (319). Moreover, EDNRB mutations can also delay the arrival of ENCCs in distal intestinal compartments, which, similar to some RET mutations, restrict the ability of ENCCs to invade and colonize distal gut compartments (258). Furthermore, it has been established that haploin sufficiency of treacle ribosome biogenesis factor (Tcof)-1 alone in mice reduces the population vagal NCCs and delays their migration along the length of the gut during early development, although it is not sufficient to provoke aganglionosis, such us in HSCR. Heterozygosity of paired box gene (Pax)-3 does not result in ENS defects either. However, if Tcof-$1^{+/-}$ is combined with a coexisting heterozygogosity of Pax-3, mice present colonic aganglionosis with cumulative apoptosis of neural crest cells leading to consequent reduction of the population of migrating ENCCs into the foregut and diminishing of the proliferating capacity of the remaining ENCCs (320).

A variety of other factors have also been proposed as possible contributors to the defects in HSCR, based on recent research. The lack of zinc finger protein X-linked (ZFX) h1B (321) or PHOX-2B (322), the inhibition of BMPs (323), the mutation of Sox-10 (324) and abnormal signaling via RA (325) are some of these factors.

10. The embryological hypothesis of colorectal carcinogenesis

Colorectal cancer (CRC) is the third most frequently diagnosed neoplasm, being the second cause of high mortality related to cancer, with the majority of cases (95%) being sporadic (326). The principles of developmental biology provide novel insight into CRC carcinogenesis and progression, creating unconventional theories regarding its origin, since human embryonic cells present major phenotypic similarity with cancer cells (327).

As regards the genetic origins of cancer, particularly CRC, the prevailing theory states that somatic mutations alter the colonic epithelium, triggering carcinogenesis (328). However, these mutations are not evenly distributed in the genome, forming complex 'mutational landscapes', which are mostly determined by the epigenetics (329). Furthermore, during cell differentiation in embryogenesis, various patterns of gene expression are defined by cardinal epigenetic alterations (330). Notably, different populations of stem cells are characterized by different epigenomic patterns (331). The above-mentioned observations suggest that a genomic mutational landscape related to CRC includes information regarding the epigenomic organization and identity of its origin cell, along with the reliable process of its embryogenesis. Nevertheless, the current knowledge regarding the oncogenic activation of specific genes related to CRC pathogenesis and their embryonic origin is very limited, relying mostly on theories.

In order to link cell differentiation and embryology, Pierce proposed that the cause of carcinogenesis could be related to development (332). According to his hypotheses, the phenotype of cancerous cells should be encoded in normal cell genome, indicating that the development of normal tissue is similar to tumorigenesis (e.g., colonic epithelium and CRC) (333). Studies have revealed that carcinogenesis can derive from the awakening of repressed genes that are normally active during embryogenesis, particularly in gastrulation, suggesting that the CRC transcriptome impersonates an embryologically active gene network as a 'developmental signature' (334). This phenomenon has been identified in several human tumors, including CRC (334,335). Another aspect that should be taken into consideration is the occurrence of keystone events during gastrulation; epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET) (336). These two processes are able to regulate cell proliferation, differentiation, invasion and migration, which are also natural traits of cancer cells during metastasis. Migratory mesenchymal cells respond differently to microenvironments adjacent to the primitive streak, resulting in the alteration of their epigenome (337), altering the transcriptional control (338), permitting the differentiation of endodermal and mesodermal cell lineages. Additionally, endodermal cells present the capacity of remodeling the basal membrane, suggesting a higher invasiveness of tumors of endodermal origin (339), and therefore a poorer prognosis compared to cancers of mesodermal or ectodermal origin. Thus, the future cancerogenic potential of tumors, such as CRC, will be proportionate to the capacity of basal membrane remodeling in response to various embryogenic signals directed by epigenomic signatures produced during gastrulation.

Since cancer may be an impaired demonstration of the physiological tissue renewal process, the role of cancer stem cells (CSCs) in carcinogenesis and development should also be considered. The current hypothesis regarding colonic CSCs states that an initial somatic mutation occurs in a normal colonic stem cell at the bottom of the colonic crypt. Following this mutational transformation, these stem cells divide in a symmetrical or asymmetrical manner producing other progenitors and CSCs, colonizing the entire niche. Furthermore, the accumulation of various alterations eventually concludes in CRC tumorigenesis and progression (340). To further investigate the morphological features of colonic stem cells, Gostjeva *et al*, through colonic tissue sampling that preserves cell architecture (341), demonstrated unique bell-shaped nuclei, which were extremely uncommon in adult normal colon, although they were abundant in fetal colon,

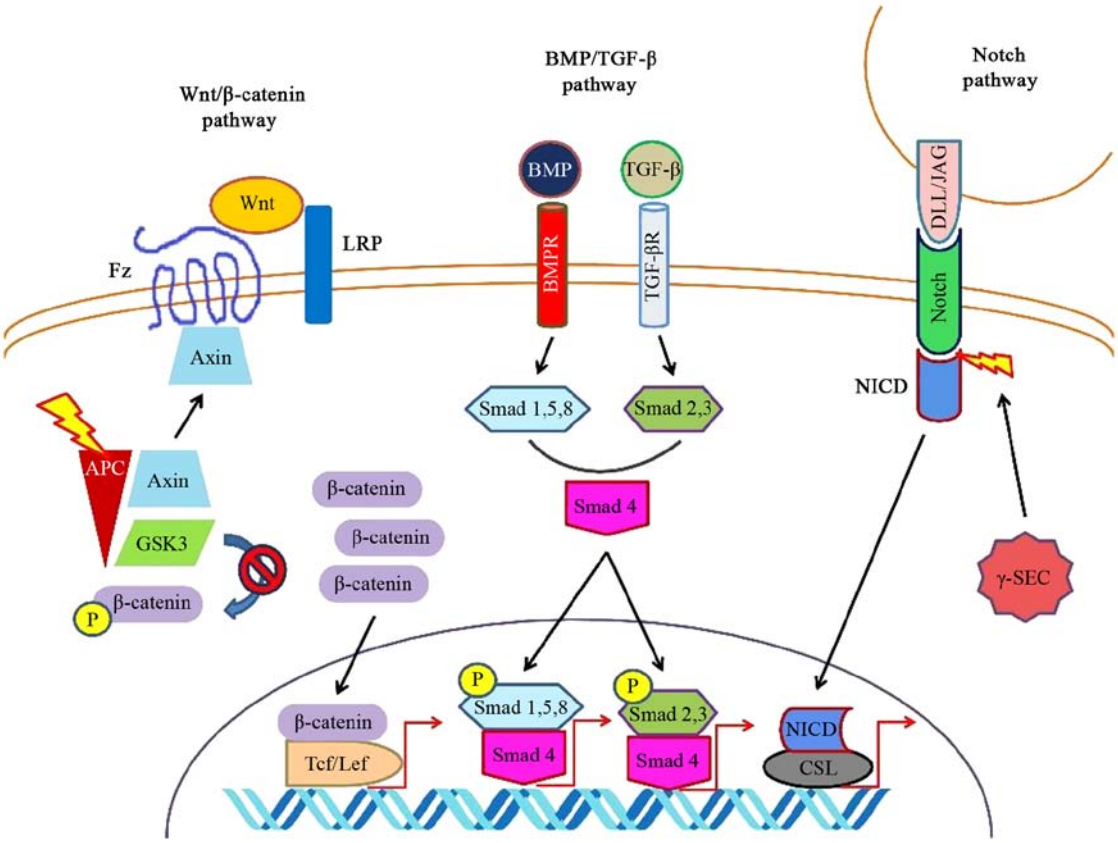

Figure 2. Common developmental pathways in gut embryogenesis and colorectal cancer. Mutations in APC result in instability of the APC-axin-GSK3 destruction complex, which insufficiently targets β-catenin for degradation after phosphorylation. Thus, increased levels of β-catenin accumulate in the nucleus, forming complexes with Tcf/Lef proteins. BMP or TGF-β bind to their receptors (BMPR and TGF-βR) and activate Smad 1,5,8 and Smad 2,3, respectively. Combined with Smad 4, they form transcriptional complexes which translocate to the nucleus. Ligands of Notch (DLL/JAG) enhance proteolysis through γ-SEC, releasing the activated NCID which translocates into the nucleus and subsequently binds with CSL. All the above signaling pathways upregulate the expression of target genes, resulting in the dysregulation of colonic stem cells and increasing their carcinogenic potential. APC, adenomatous polyposis coli; BMP, bone morphogenetic protein; BMPR, bone morphogenic protein receptor; CSL, CBF-1, Suppressor of Hairless, Lag-1; DLL, delta-like; Fz, frizzled; GSK, glycogen synthase kinase; JAG, jagged; Lef, lymphoid enhancer-binding factor; LRP, low-density lipoprotein receptor-related protein; NICD, notch intracellular domain; Tcf, T-cell factor; TGF, transforming growth factor; TGF-βR, transforming growth factor-beta receptor; Wnt, wingless-related integration site; γ-SEC, γ-secretase.

colonic adenomas and adenocarcinomas (342). In CRC, such nuclei were mainly detected in the center of the tumoric mass and at the bottom of cancerous crypts. These results support the hypothesis of embryonic basis of CRC carcinogenesis, which is depended on colonic stem cells via transformation to a more juvenile proliferation state, turning them into CSCs and finally leading to CRC. A very recent theoretical model tries to unify the CSC and embryonic hypothesis in the context of carcinogenesis based on the principles of gastrulation (343). According to this proposal, a normal adult differentiated cell can transform into a CSC, after undergoing deprogramming (dedifferentitation) and reprogramming in the lack of genomic stability. This CSC can be considered as a para-embryonic stem cell, which actually constitutes the initial CSC (CSC0) in the process of tumorigenesis. The CSC0 can generate a primary tumor, which resembles a pre-implantation blastocyst. In a suitable niche, regarding signaling factors, this primary tumor would be implanted, resembling a post-implantation blastocyst. From these cells, pluripotent, slow self-renewing primary CSCs (CSC1s) would be produced. CSC1s resemble the epiblast cells, and being epigenetically blocked in this primed state would act as tumor-initiating cells. CSC1s would then produce secondary CSCs (CSC2s) resembling hypoblast cells, which would act as tumor-growth cells. CSC1s or CSC2s would produce tertiary CSCs (CSC3s), possessing a mesenchymal phenotype, which would act as tumor-migrating cells, mimicking the mesodermal precursors at the primitive streak. If the microenvironment of the primary tumor creates preferable conditions such as normoxia, the CSC3s will then proceed into asymmetrical division creating cancer progenitor cells (CPCs), which then would differentiate into cancer differentiated cells (CDCs), thus completing a defined cell hierarchy resembling somito-histo-organogenesis. However, unfavorable conditions, such as hypoxia, would trigger the migration/delamination of CSC3s, behaving as migrating micrometastases, corresponding to the mesenchymal cells during gastrulation. In distant metastatic niches, CSC3s would be localized in a dormant state as an effect of hypoxia and EMT signaling. Contrarily, when normoxia or MET signaling prevail in the metastatic niche, a CSC3/CSC1 reversion would then be induced, and the newly formed CSC1s, as tumor-initiating cells would reproduce the same cell hierarchy of the primary tumor as macro-metastases (343).

Various embryonic molecular signaling pathways are emerging as major determinants of CRC carcinogenesis and progression, being common between embryogenesis

Table II. Summary of the expression and function of molecular factors that regulate embryogenesis in the large intestine.

Molecular factors	Site of expression	Functions
Activin	Former mesendoderm	- Endoderm formation
	Splanchnic mesoderm	- LR pattern control
BMP	Mesenchymal mesoderm	- Smooth muscle formation (BMP-4)
		- Maturation of enteric neurons (BMP-2)
	Subepithelial mesenchyme	- RAD pattern control
		- Crypt formation
		- Epithelial and mesenchymal development and differentiation (BMP-2,4,7)
	Mesenchyme	- ENCC migration, survival, differentiation
		- Enteric ganglia formation (BMP-2,4)
Cdx	Posterior endoderm	- Hindgut formation and differentiation (Cdx-2)
Chato	Primitive endoderm	- Endoderm elongation
Dact	Posterior endoderm	- Hindgut and CIP formation (Dact-1)
Elf	Epithelium	- Villi formation (Elf-3/Crif-1 through TGF-β)
Epimorphin	Mesenchyme	- Secretion of molecules for endodermal differentiation
ET	Mesenchyme	- ENCCs proliferation, inhibition of cytodifferentiation (ET-3 through EDNR-B)
FGF	Endoderm	- AP pattern of hESCs (FGF-2)
	Posterior endoderm	- Hindgut formation (FGF-4)
	Epithelium	- Proliferation and elongation of mesenchyme (FGF-9)
	Mesenchyme	- Formation of cecal budding (FGF-10)
	Splanchnic mesoderm	- LR pattern control (FGF-8)
Fox	Former mesendoderm	- Endoderm formation of foregut and midgut (Fox-A)
	Anterior endoderm	- AP pattern control (Fox-A2)
	Subepithelial mesenchyme	- Epithelial organization and maturation, RAD pattern control
		- Mesenchymal maturation
		- Villi formation
		- Crypt formation (Fox-l1, Fox-F1, Fox-F2)
	Mesenchyme	- ENCCs migration through modulation of ECM (Fox-F1, Fox-F2)
GATA	Former mesendoderm	- Endoderm formation
	Early definitive endoderm of AIP	- Endoderm invagination (GATA-4)
GDNF	Mesenchyme	- ENCC proliferation, migration, and survival (through RET/PI3K activation)
Gli	Subepithelial mesenchyme	- Epithelial organization and maturation, RAD pattern control
		- Mesenchymal maturation
		- Villi formation
		- Crypt formation (Gli-2, 3)
HAND	ENCCs	- ENCCs maturation, neuro-transmitter specification (HAND-2)
HAT	Endoderm	- RAD pattern control
		- Villi formation
HDAC	Endoderm	- RAD pattern control
		- Villi formation (HDAC1,2)
Hox	Mesoderm of midgut and hindgut	- AP patterning of midgut and hindgut (Abd-B subfamily of Hox-A and D clusters)
Isl	Left side splanchnic mesoderm	- LR pattern control (Isl-1)
L1CAM	ENCCs	- ENCCs migration through interaction with ECM
MMP	ENCCs	- Migration through degradation of ECM (MMP-2)
Netrin	Epithelium	- Direction of axon formation
		- ENCCs migration through RAD axis
Nodal	Former mesendoderm	- Endoderm differentiation (high signaling)
		- Mesoderm differentiation (low signaling)
	Splanchnic mesoderm	- LR pattern control

Table II. Continued.

Molecular factors	Site of expression	Functions
Notch	Endoderm	- Epithelial cytodifferentiation (through Hes-1, Atoh-1, and crosstalk with Wnt/β-catenin)
PDGF	Endoderm	- RAD pattern control - Epithelial and mesenchymal maturation - Crypt and villi formation (PDGF-A)
PHOX	ENCCs	- ENCCs proliferation, survival - Enteric ganglia formation - Neuronal formation (PHOX-2B)
Pitx	Left side splanchnic mesoderm	- LR pattern control (Pitx-2, through Shroom3, N-cadherin)
RA	Paraxial mesoderm	- Directing NCCs into the formation of ENS (through RET)
	Mesoderm	- AP pattern control
	Mesenchyme	- ENCCs proliferation, migration (through inhibition of PTEN)
Shh/Ihh	Endoderm of AIP and CIP	- Endoderm invagination - Mesodermal formation
	Splanchnic mesoderm	- LR pattern control
	Endoderm	- Villi formation - Crypt formation - Maturation of mesenchyme and epithelium
	Epithelium	- ENCCs migration through RAD axis (through PTC-1)
Sox	Former mesendoderm	- Endoderm formation of midgut and hindgut (Sox-17)
	Anterior endoderm	- AP pattern control (Sox-2)
	ENCCs	- ENCC survival, proliferation, inhibition of cytodifferentiation - Glial cell formation (Sox-10)
T-box	Former mesendoderm	- Endoderm formation
	Right side splanchnic mesoderm	- LR pattern control (Tbx-18)
Wnt/Ca^{2+}	Mesoderm	- Elongation of mesenchyme and ventral development (through Wnt5a and Fz2)
Wnt/PCP	Epithelium	- Cytoskeleteal organization - Apical-basal epithelial polarization, RAD pattern control (through Wnt5a, Ezrin) - Crypt development
	Mesoderm	- Elongation of mesenchyme (through Wnt11 and Fz7)
Wnt/β-catenin	Endoderm	- AP pattern control (through Tcf-1,4 and Lef-1) - Epithelial proliferation and cytodifferentiation - Villus formation (through Tcf-3,4) - Crypt formation (through Ephrin-B)

Abd, abdominal; AIP, anterior intestinal portal; AP, antero-posterior; Atoh, atonal homolog; BMP, bone morphogenetic protein; Cdx, caudal-related homeobox; CIP, caudal intestinal portal; Crif, CR6-interacting factor; Dact, dapper; ECM, extracellular matrix; EDNR, endothelin receptor; Elf, E74-like factor; ENCCs, enteric neural crest cells; ENS, enteric nervous system; ET, endothelin; FGF, fibroblast growth factor; Fox, forkhead-box; Fz, frizzled; GDNF, glial cell-derived neurotrophic factor; Gli, glioma-associated oncogene homolog; HAND, heart- and neural crest derivatives; HAT, histone acetyltransferase; HDAC, histone deacetylase; Hes, hairy and enhancer of split; hESCs, human embryonic stem cells; Hox, homeobox; Ihh, indian hedgehog homolog; Isl, islet; L1CAM, L1 cell adhesion molecule; Lef, lymphoid enhancer-binding factor; LR, left-right; MMP, matrix metalloproteinase; NCCs, neural crest cells; PCP, planar cell polarity; PDGF, platelet-derived growth factor; PHOX, paired-like homeobox; PI3K, phosphoinositide 3-kinase; Pitx, paired-like homeodomain transcription factor; PTC, patched homolog; PTEN, phosphatase and tensin homolog; RA, retinoic acid; RAD, radial; RET, rearranged during transfection; Shh, sonic hedgehog; Sox, SRY-related HMG-box; Tbx, T-box; Tcf, T-cell factor; TGF, transforming growth factor; Wnt, wingless-related integration site.

and cancer (Fig. 2). The canonical Wnt/β-catenin signaling pathway is one of the most commonly involved in cancer. In the vast majority of sporadic CRC cases (90%) a mutation in the tumor suppressive APC gene as the initial molecular change, leading to the loss of expression of the corresponding protein and to the hyperstimulation of the Wnt/β-catenin

Table III. Molecular factors and related disorders in intestinal embryogenesis.

Molecular factors	Disorders
BMP	- (General inhibition) polyp formation, larger and fewer villi, less compact subepithelial mesenchymal development - (BMP-2,4,7 misexpression/mutation) impairment of all layer development and differentiation, disruption of RAD pattern - (BMP-2 overexpression) decreased survival of gut neural cells - (BMP-2,4 inhibition) disruption of ENCCs migration, imbalanced neural-glial ENCC differentiation, HSCR - (BMPR1 misexpression/mutation) juvenile polyposis
Canonical Wnt pathway	- (General inhibition) loss of epithelial proliferation, depletion of progenitor cells, arrest of secretory cell cytodifferentiation - (Tcf-1,4 knockout) disruption of AP pattern, loss of caudal hindgut region, altered differentiation of duodenum, endodermal defects, CIP formation failure, loss of Fox-A1, Sox-17 and Shh expression in hindgut, midgut disclosure - (Lef-1 knockout) cecal stenosis, posterior mesodermal disorders - (Wnt3a,5a lack) posterior mesodermal disorders - (Ascl2 deletion) inhibited stem cell replication, crypt diminishment
Cdx	- (Cdx-1,4 lack) disruption of AP pattern, absence of intestinal phenotype - (Cdx-2 loss) disruption of AP pattern, disoriented endodermal differentiation, absence of intestinal phenotype, alteration of gut epithelium to esophageal epithelium - (Cdx-2 misexpression/mutation) colonic atresia, disruption of RAD pattern
Chato	- (Lack) ineffective endodermal elongation, unsuccessful gut tube closure
Dact-1	- (Knockout) CIP formation failure, ventral endodermal folding failure, hindgut and cloaca formation failure
DCC	- (Misexpression/mutation) failed development of submucosal neural plexus
EGFR	- (Deletion) delayed villus emergence, diminished epithelial proliferation, villus blunting, tissue disintegration
Elf-3/Crif-1	- (Knockout) diminished TGF-βRII expression, fewer malformed villi, lamina propria disorganization, epithelial malfunction
Epimorphin	- (Misexpression/mutation) disruption of RAD axis, inhibition of epithelial morphogenesis, acceleration of epithelial proliferation, disruption of BMP and Hedgehog pathways
ET	- (ET-3, EDNRB, ECE-1 deletion) colorectal aganglionosis, HSCR
FGF	- (FGF-12 improper function) heterotaxy syndrome, disruption of LR pattern - (FGF-10 misexpression/mutation) colonic and duodenal atresia, disruption of RAD pattern - (FGFR2IIIb knockout) intestinal atresia, disruption of RAD pattern, altered RA signaling

Table III. Continued.

Molecular factors	Disorders
Fox	- (Fox-A2 improper function) heterotaxy syndrome, disruption of LR pattern - (Fox-F1 misexpression/mutation) intestinal atresia, disruption of RAD pattern - (Fox-L1 misexpression/mutation) reorganization of epithelium and villi, deregulated crypt development and branching - (Fox-F1,F2 silencing) mesenchymal disintegration after villi formation
GALNT11	- (Improper function) heterotaxy syndrome, disruption of LR pattern, disruption of left side nodal flow
GATA-4	- (Knockout) AIP malformation, foregut absence, yolk sac displacement, defective lateral-ventral folding
HAND-2	- (Deletion) fewer enteric neurons, disrupted ENCCs differentiation
HAT	- (Misexpression/mutation) delayed villus emergence, failed subepithelial mesenchymal condensation, decreased BMP-4 expression
HDAC	- (Overexpression) blocked epithelial differentiation - (Inhibition) immature villus development, abnormal epithelial differentiation
Hox	- (Hox-A13 misexpression/mutation) disruption of AP pattern, protein truncation, endodermal defects - (Hox-D13 misexpression/mutation) disruption of AP pattern, hindgut differentiation of midgut epithelium
Ihh	- (Misexpression/mutation) decreased epithelial proliferation, reduced size and number of villi
Inversin	- (Improper function) disruption of forward cilial movement, situs inversus
Isl-1	- (Ectopic expression) disruption of LR pattern, bilateral symmetry, bilateral Pitx-2 expression, loss of right-sided Tbx-18 expression
KIF	- (KIF3B lack) ciliogenesis impairment, disruption of LR pattern, prenatal death, situs inversus - (KIF3A loss) diminishment of nodal mechanosensory ability, disruption of LR pattern, situs inversus
L1CAM	- (Inhibition) ENCCs close contact abruption, slow ENCCs migration
MMP-2	- (Inhibition) reduced ENCCs migration
Nkx2.3	- (Misexpression/mutation) mesenchymal cell reduction, decreased epithelial proliferation, delayed villus emergence, intrauterine death, epithelial hyperproliferation, mucosal thickening, branched villi, abnormal epithelial architecture
Nodal	- (Ectopic expression) disruption of LR pattern, bilateral symmetry
Non-canonical Wnt pathway	- (Wnt5a knockout) shorter intestinal length, deregulated apical-basal polarity of intestinal epithelium - (sFRP improper function) shorter intestinal length, deregulated apical-basal polarity of intestinal epithelium - (Dvl-1,2,3 improper function) PCP deregulation, disruption of LR pattern, situs inversus

Table III. Continued.

Molecular factors	Disorders
	- (Ezrin absence) improper epithelial polarization, villus fusion, mucosal disorganization
Notch	- (Atoh-1 misexpression/mutation) inhibition of secretory cytodifferentiation, abnormal epithelial proliferation
PDGF	- (Inhibition) disrupted colonic mucosal architecture
PHOX-2B	- (Misexpression/mutation) total agangliosis, enhanced glial differentiation, HSCR
Pitx-2	- (Ectopic expression) disruption of LR pattern, bilateral symmetry, symmetrical bilateral Isl-1 expression, loss of right-sided Tbx-18 expression - (Knockout) loss of Isl-1 expression, bilateral expression of Tbx-18, bilateral symmetry
Pkd-2	- (Loss) diminishment of nodal mechanosensory ability, disruption of LR pattern, situs inversus
RA	- (Raldh-2 deficiency) ENS agenesis, decreased RET expression - (abnormal signaling) HSCR
RET-GDNF	- (RET knockout) total agangliosis - (RET misexpression/mutation) HSCR - (GDNF insufficiency) HSCR - (PI3K inhibition) distal agangliosis, limited ENCCs migration - (SPRY-2, KIF26A silencing) enteric nerve hyperplasia
Shh	- (Knockout) esophageal malformation, foregut mesodermal absence, defective mesenchymal development, defective epithelial maturation, disrupted villi organization, disruption of RAD pattern, ectopic submucosal ganglia formation, malformed neural projections - (Overexpression) mesodermal overdevelopment, intestinal agangliosis, inhibited ENCCs migration - (misexpression/mutation) intestinal atresia, villi overgrowth
Sox-10	- (Misexpression/mutation) decreased ENCCs survival, premature ENCCs differentiation, distal agangliosis, HSCR

AIP, anterior intestinal portal; AP, antero-posterior; Ascl, achaete-scute complex homolog; Atoh, atonal homolog; BMP, bone morphogenetic protein; BMPR, BMP receptor; Cdx, caudal-related homeobox; CIP, caudal intestinal portal; Crif, CR6-interacting factor; Dact, dapper; DCC, deleted in colorectal cancer; Dvl, Dishevelled; ECE, endothelin converting enzyme; EDNR, endothelin receptor; EGFR, epidermal growth factor receptor; Elf, E74-like factor; ENCCs, enteric neural crest cells; ENS, enteric nervous system; ET, endothelin; FGF, fibroblast growth factor; FGFR, FGF receptor; Fox, forkhead-box; GALNT, N-acetylgalactosaminyltransferase; GDNF, glial cell-derived neurotrophic factor; HAND, heart- and neural crest derivatives; HAT, histone acetyltransferase; HDAC, histone deacetylase; Hox, homeobox; HSCR, Hirschsprung disease; Ihh, indian hedgehog homolog; Isl, islet; KIF, kinesin family member; L1CAM, L1 cell adhesion molecule; Lef, lymphoid enhancer-binding factor; LR, left-right; MMP, matrix metalloproteinase; Nkx2.3, NK2 homeobox 3; PCP, planar cell polarity; PDGF, platelet-derived growth factor; PHOX, paired-like homeobox; PI3K, phosphoinositide 3-kinase; Pitx, paired-like homeodomain transcription factor; Pkd, polycystin; RA, retinoic acid; RAD, radial; Raldh, retinaldehyde dehydrogenase; RET, rearranged during transfection; sFRP, secreted frizzled-related protein; Shh, sonic hedgehog; Sox, SRY-related HMG-box; SPRY, Sprouty homolog; Tbx, T-box; Tcf, T-cell factor; TGF-βR, TGF-β receptor; Wnt, wingless-related integration site.

pathway (344). Although canonical Wnt components are of major importance for the regulation of the integrity of normal stem cells and CSCs, the deregulation of β-catenin levels is not solely sufficient for CRC carcinogenesis. Specifically,

APC mutation creates a basal impairment of β-catenin activity and then further genetic alterations induce the hyper-stimulation of Wnt/β-catenin signaling pathway, leading to the production of colonic cells with CSC characteristics and enhanced metastatic capacity (345). Moreover, the canonical Wnt signaling pathway through positive or negative cross-talk with other developmental pathways, including PI3K, Notch, BMP and Hedgehog can regulate the CSC population (346). Notably, components of the non-canonical Wnt signaling pathways seem to be involved in the association between colon embryogenesis and CRC carcinogenesis. The detection of methylated DNA by Kaiso, via methyl-CpG binding location, is crucial for the epigenomic silencing of tumor-suppressive genes, an essential role that has already been described in CRC cancerogenesis (347). The nuclear translocation of Kaiso/p120ctn stabilizes cells that have already undergone MET, reduces MMP-7 gene expression along with cell migration, and triggers E-cadherin expression, ultimately inducing the endodermal formation including the primitive colon (348), thus functioning as an epigenetic control system connecting CRC and embryogenesis. The TGF-β/BMP signaling pathway is another essential pathway in both development and cancer, promoting cell migration and invasion (349). Despite their compartmentalized activity in the normal colonic tissue, the TGF-β/BMP and Wnt/β-catenin signaling pathways are altered in CRC, crosstalking in inducing CRC carcinogenesis (350). BMP-2 and BMP-4 in particular participate in the regulation of colonic CSCs by furthering CSC differentiation and antagonizing the canonical Wnt signaling (351). Finally, concerning the molecular basis of EMT, during embryogenesis, both Wnt and TGF-β signaling pathways regulate the formation of the primitive streak through induction of EMT by upregulating SNAIL and TWIST transcription factors (352). The same EMT regulators also promote EMT and consequent migration and metastasis in CRC (353,354). Notch signaling pathway is another link between embryogenesis and CRC carcinogenesis, acting as a controller of colonic CSCs, since it is crucial for the homeostasis of the normal colon stem cell (355). Evidence demonstrates the control of stem cell expansion and self-renewal by a signaling consortium of SNAIL, miR-146a, and Numb, a Notch inhibitor, leading to fine-tuning of Wnt signaling (356).

11. Conclusions

Large intestine embryogenesis constitutes an extremely complex procedure, involving a vast plethora of cellular populations cross-talking through various ways. These interactions are mediated and controlled via numerous molecular pathways (Table II). However, deregulation may occur, resulting in developmental disorders which could be fatal in embryonic life, or cause long-term issues in adult life (Table III). Moreover, novel insight into these pathways reveals a possible connection between embryology and carcinogenesis. Future studies are required to focus on further investigating and clarifying the detailed role of the molecular components in the development of large intestine and related pathological conditions.

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Authors' contributions

JT conceived and designed the study. AK, IK and KN researched the literature, processed the figures and drafted the manuscript. KN, DAS, AT and JT edited the manuscript, assisted in the literature search, critically revised the article for important intellectual content and provided the final approval of the version to be published. All authors have read and approved the final manuscript.

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Competing interests

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