Control of cell adhesion and compartmentalization in the intestinal epithelium

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Summary

Continuous cell renewal in the intestinal mucosa occurs without disrupting the integrity of the epithelial layer. Despite the restrictions imposed by strong cell-to-cell adhesions, epithelial intestinal cells migrate constantly between tissue compartments. Alterations in cell adhesion and compartmentalization play key roles in diseases of the intestine. In particular, decreased E-cadherin-mediated adhesion during inflammatory bowel disease and loss of EphB/ephrin-B-mediated compartmentalization in colorectal cancer have recently emerged as key players of these prevalent pathologies. Here we will review our current knowledge on how cell-to-cell adhesion, migration and cell positioning are coordinated in the intestinal epithelium. We will highlight what the *in vivo* genetic analysis of intestinal epithelium has taught us about the complex regulation of cell adhesion and migration in homeostasis and disease.

Keywords

Intestinal epithelium, E-cadherin, p120-catenin, EphB receptors, Rho GTPases, colorectal cancer, inflammatory bowel disease.

Introduction

Most differentiated cell types of the intestinal epithelium have a short lifespan (3 to 5 days) and therefore must be constantly renewed from undifferentiated progenitor cells. Cell renewal in the intestinal epithelium is orchestrated through continuous cell migration from the crypts towards the luminal surface. The origin of the migratory cell column is the crypt base around position +4 which represents the boundary between the Intestinal Stem Cell niche and the Transient Amplifying compartment. The vast majority of progenitor cells migrates upwards and undergoes cell cycle arrest and differentiation as they reach the surface epithelium. Upward migrating cells differentiate mainly into absorptive or mucosecreting phenotypes and eventually are shed into the lumen at the villi tips. An exception to the upward migratory flow are Paneth cells which move downwards and localize to the crypt base intermingled between ISCs. Maturation of Paneth cells occurs as they move towards the bottommost positions. Enteroendocrine and tuft cells represent less than 1% of all epithelial cells and reside scattered along the crypts and the villus. It is unclear whether they escape the migratory flow or perhaps undergo differentiation at different positions of their migratory path. The intestinal epithelium not only performs absorption of nutrients but also acts as a robust barrier separating the harsh environment of the intestinal lumen from the underlying tissues. Thus, the formation of strong adhesions between epithelial cells as well as the establishment of cell compartment boundaries must be intimately coupled to the migratory process.

Control of cell-to-cell adhesion during intestinal homeostasis.

The integrity of the epithelial layer is maintained by a strong network of cell-to-cell adhesions which includes adherens (AJs) and tight junctions (TJs). The adhesion molecules that nucleate AJs are the cadherins which are single pass transmembrane proteins that form homotypic calcium dependent adhesions with cadherins from neighboring cells. Manipulation of E-cadherin function in the intestinal epithelium has revealed important roles not only in the formation of cell adhesions but also in the control of cell signaling. Conditional ablation of E-

cadherin in the intestinal epithelium of mice caused rapid death due to massive diarrhea as a result of enhanced apoptosis and cell shedding. E-cadherin null epithelium displayed a block in cell differentiation and defects in cell polarization, in desmosomal complex stability and in cell-to-matrix adhesion [1]. On the other hand, forced expression of E-cadherin in intestinal epithelial cells slowed down the migration rate without disturbing the differentiation programs [2]. Experiments where N-cadherin was knocked into the E-cadherin locus revealed additional functions of E-cadherin besides its role in adhesion [3]. The intestine of mice with E-cadherin to N-Cadherin substitution developed normally and did not display apparent defects in cell adhesion [3]. Yet, after birth, these mice developed an expansion of the crypt domain and dysplasia as a result of enhanced Wnt signaling due to increased nuclear localization of beta-catenin [3]. This phenotype may be explained by the inability of N-cadherin to quench beta-catenin, perhaps as a result of E-cadherin and N-cadherin exhibiting different affinities for beta-catenin. Alternatively, E-cadherin or N-cadherin may crosstalk with distinct signaling pathways. The so-called cadherin switch (i.e. silencing of E-cadherin expression and upregulation N-cadherin levels) is a common process during tumor progression and confers tumor cells with enhanced motility and proliferation capacity [4]. This effect is in part due to differential interactions of both types of cadherins with growth factor receptors [4].

Other components of the AJs complex have been deleted in the intestinal epithelium of mice. Beta-catenin is a core component of AJs. It has been long believed to serve as bridge between the intracellular tail of E-cadherin and alpha-catenin/actin cytoskeleton. Yet, this view has been challenged by the discovery that alpha-catenin does not bind E-cadherin/beta-catenin complexes and actin filaments simultaneously [5]. In addition, beta-catenin acts as the nuclear partner of the TCF family of transcription factors during the transduction of Wnt signals. Beta-catenin deletion in the intestinal epithelium did not cause major defects in cell-to-cell adhesion but rather it resulted in loss of the crypt compartment and alterations

in the differentiation process [6-7]. These phenotypes are likely due to impaired Wnt signaling which is essential for intestinal stem cell maintenance.

p120-catenin is required to stabilize E-cadherin [8]. KO mice for p120-catenin in the intestine exhibited cell-to-cell adhesion defects which led to progressive mucosal erosion and terminal bleeding [9]. Whereas this phenotype is somewhat reminiscent of that of E-cadherin null intestine [1], other alterations observed in these animals are hardly attributable to AJs malfunction. p120-catenin has been proposed to act as a GDP-dissociation inhibitor (GDI) for the RhoA small GTPase, keeping RhoA in its inactive state in the cytoplasm of cells [8]. This observation was consistent with the increased activation of RhoA effectors (i.e. Phospho-Ezrin) observed in p120-catenin KO intestinal mucosa [8]. In addition, p120-catenin null epithelium displayed enhanced expression of stress-markers and recruitment of neutrophiles which links p120-catenin deficiency with inflammation (see below). The latter observations are compatible with the phenotype described for mice null for p120-catenin in the skin which showed activation of RhoA and onset of inflammatory response due to aberrant NF-κB activation [10].

Small GTPases of the Rho family (RhoA, Rac1 and cdc42) play a central role in coupling AJs with the cytoskeleton. Rho GTPases control AJs formation and stability [11]. In vitro, RhoA decreases AJs stability whereas Rac1 and cdc42 appear to have the opposite role [11]. In turn, a feedback loop exists by which Rho GTPase activity is also controlled by AJs. RhoA functions in the intestine are poorly understood due to lack of *in vivo* models. However, the Rho effector molecule Ezrin has been investigated in more detail [12]. Ezrin belongs to the ERM (Ezrin, Radesin, Moesin) protein family and serves as an intermediate between the plasma membrane and the actin cytoskeleton. It is the only ERM expressed in the intestine [12]. Mice mutant for Ezrin displayed developmental alterations in the transition from a stratified intestinal epithelium to a columnar epithelium characteristic of the mature gut. As a result, mice were born with fused and rounded villus. This defect was probably due to lack of cytoskeletal tension at the

apical domains of villus enterocytes which is required for secondary lumen morphogenesis. Junctional complexes were also disturbed in intestinal epithelial cells lacking Ezrin [12].

The role of Rac1 has also been addressed *in vivo* [13]. Transgenic expression of a constitutively activated form of Rac1 (Rac1Leu61) in the intestinal epithelium driven by the proximal promoter of the Fatty acid binding protein 1 (-596 to +21 - Fabp1) caused lethality at post-natal day 1 [13]. In late embryonic stages, Rac1Leu61 expression led to premature maturation of Paneth cells and enterocytes. Defects in reorganization of the apical actin cytoskeleton were only observed in the intervillus epithelium (presumptive crypts) but not in the villi. However, these mice did not display major alterations in E-cadherin or β-catenin levels. Conversely, mice expressing a dominant negative version of Rac1 (Fabp1-Rac1Asn17) developed elongated crypts and displayed a block in the differentiation of Paneth and mucosecreting cells [13]. Rac1Asn17 overexpression also caused delayed migration of cells along the crypt axis [13]. Of note, ablation of Rac1 in skin epithelium induced loss of hair follicle stem cells [14]. In light of this result, and given the possibility that the Fabp1 promoter did not drive strong transgene expression in ISCs, the effects of Rac1 activation or inhibition in the intestinal epithelium deserve further investigation.

Finally, Tiam1, a selective Rac GTPase activator, is a Wnt-target gene expressed at the crypt base as well as in APC mutant adenomas [15]. Deletion of Tiam1 in the intestinal epithelium did not result in apparent abnormalities in homeostasis yet it reduced tumor burden caused by mutations in APC. This observation implies that Tiam1 is required for early stages of Wnt-driven tumor formation. However, adenomas developed by compound Tiam1; ApcMin/+ mice became invasive more frequently compared to those of ApcMin/+ mice littermates which remained largely benign [15]. These observations suggest opposite roles for Tiam1 during CRC initiation and progression. It is unclear whether these effects are related to or are independent of Rac1 activation.

Tight Junctions form a belt-like adhesion which seals the epithelium as well as regulates the selective flow of molecules in the intercellular space (i.e. paracellular permeability) [16]. Whereas the role of TJs in controlling epithelial permeability has been widely explored using *in vitro* cell models, few TJ components have been deleted *in vivo*. Two of such proteins are claudin-2 and claudin-15. Claudins form a large family of proteins characterized by the presence of four transmembrane domains. They are key mediators of paracellular permeability. Mice deficient in claudin-15 developed enlarged intestines due to increased proliferation of crypt progenitor cells after weaning [17]. These mice also displayed defects in paracellular permeability of Na+ ions [18]. Because the transport of glucose and sodium in the small intestine are intimately coupled, Claudin-15-/- mice also developed deficiency in the absorption of glucose [18]. On the contrary, Claudin-2 deficient mice had a normal-sized intestine despite displaying defects in paracellular permeability in infant mice which became less pronounced in adult intestine [18]. It is unclear whether increased crypt proliferation in Claudin-15 null mice is a compensatory reaction induced by impaired absorption or if Claudin-15 may also directly control the division rate of progenitor cells. It is also interesting to point out that during the development of the zebrafish gut, Claudin-15 is essential for the coalescence of multiple small lumens into the single lumen characteristic of the mature intestine [19]. This phenomenon was not directly connected to the adhesive function of TJs but rather to the control of paracellular permeability. Pores created by Claudin-15 allowed the asymmetric distribution of ions between the luminal and paracellular space which induced enlargement of small lumens due to accumulation of fluids. Enlarged lumens eventually fused and coalesced into a single luminal space [19].

Defects in cell-to-cell adhesion associated to inflammatory bowel diseases

Although the precise cause of inflammatory bowel diseases (IBDs) such as ulcerative colitis and Crohn’s disease is not well understood, recent evidences suggest that defects in the integrity of the intestinal epithelium due to alterations in

E-cadherin function may be at the origin of these pathologies. Studies *in vivo* and *in vitro* suggest that an activated intestinal immune system in patients with IBD is the result of a raised local antigen exposure associated with an increased permeability of the intestinal epithelial layer [16]. Loss of normal membranous E-cadherin, alpha-catenin and p120-catenin have been detected at the mucosal edges around epithelial ulcerations in all cases of active ulcerative colitis and in 50 per cent of cases with active Crohn's disease [20]. Expression of a dominant negative form of N-cadherin in the intestinal epithelium which disrupts E-cadherin based adhesions in mice resulted in lesions that resembled IBD as well as in the formation of adenomas [21]. In addition, KO mice for p120-catenin in the intestine showed both cell-to-cell adhesion defects and inflammation characterized by massive cox2+ neutrophil infiltration which is a common finding in patients with ulcerative colitis [9]. It has been hypothesized that loss of cell adhesion and increased mucosal permeability in IBDs may be simply a secondary effect of inflammation as several inflammatory mediators including TNF-alpha are capable of regulating permeability of the intestinal epithelium [16, 22]. Yet, recent genome wide association studies (GWAS) may change this view. In particular, GWAS of 2361 cases of ulcerative colitis identified variants of the genes encoding for E-cadherin (CDH1) and P-Cadherin (CDH3) associated with the disease [23]. The same CDH1 locus variant had been previously associated with risk of developing CRC [24]. Collectively these observations suggest a causative role for decreased E-cadherin-mediated adhesion in the onset and/or progression of IBD. In addition, GWAS identified HNF4A gene polymorphisms associated with ulcerative colitis [23]. Adult mice deficient for the transcription factor HFN4A in the intestinal epithelium showed increased epithelial permeability as well as increased susceptibility to intestinal inflammation upon dextran sodium sulfate (DSS) treatment [25-26] or spontaneous IBD-like disease [26]. Interestingly, the latter study identified Claudin-15 as a target of HFN4A [26]. Furthermore, single-nucleotide polymorphisms (SNP) in the Rac1 gene have recently been associated with high risk of inflammatory bowel disease in three independent cohorts of patients [27]. Carriers of the Rac1 high risk allele expressed higher levels of Rac1

[27]. Authors demonstrate that mice with a conditional deletion of Rac1 in macrophages and neutrophils were protected from DSS-induced colitis thus suggesting that Rac1 is required in immune cells during the inflammatory response associated with IBD. As Rac1 also plays a central role in the formation and stability of cell-cell junctions *in vitro* [11], it is tempting to hypothesize that increased Rac1 levels due to gene polymorphisms may also alter the barrier function of the intestinal epithelium.

Formation of cell compartments in the intestinal epithelium by EphB-ephrin-B signaling.

As commented extensively in this review issue, Wnt signaling is required to specify and maintain the stem cell compartment of the crypts as well as for the correct maturation of Paneth Cells. A few years ago, we discovered that beta-catenin and TCF also regulate cell positioning in the crypt through controlling the expression of the EphB family of receptors and their ligands, the ephrin-Bs [28]. Eph receptors comprise the largest subgroup of receptor tyrosine kinases characterized for binding to membrane-bound ligands known as ephrins [29]. With a few exceptions, there is a ligand subclass specificity in the Eph/ephrin family; that is, B-type Eph receptors bind preferentially transmembrane ephrins (B-type ephrins), whereas A-type Eph receptors bind GPI-membrane anchored ephrins (A-type ephrins) [29]. Eph receptors are well-known mediators of cell compartmentalization and guided cell migration during embryonic development [30]. They play key roles in the pathfinding of some axonal tracts and in the establishment of boundaries between adjacent cell populations in segmented structures such as the hindbrain or the somites [30]. In intestinal epithelial cells, beta-catenin and TCF promote the expression of the receptors EphB2 and EphB3 by direct binding to their promoters [28, 31]. On the contrary, Wnt signaling represses the expression of the EphB-receptor ligands ephrin-B1 and ephrin-B2 [28]. As a result of the opposite regulation of EphB receptors and ephrin-B ligands by Wnt signaling, receptors and ligands are expressed in different compartments along the crypt axis [28]. EphB2 is present at highest levels in ISCs and its expression gradually decreases in

progenitor cells as they migrate towards the lumen. EphB3 displays a restricted localization in those cells present at the bottommost positions of the crypt (i.e. Paneth cells and ISCs). Consistently with their negative regulation by Wnt signaling, the expression of ephrin-B1 increases gradually as the progeny of the ISC leaves the crypt base whereas ephrin-B2 decorates the differentiated cells of the surface epithelium and the villus [28] .

Mouse deficient in EphB2 or EphB3 display an array of defects in intestinal cell migration and compartmentalization [28]. In the intestine of EphB3 null mice, localization of Paneth Cells was no longer restricted to the crypt base. Instead, this cell type moved with the upward migratory flow and was found dispersed throughout the crypt and villus axis. This phenotype was also observed in intestine specific conditional ephrin-B1 knockout mice [32]. In double EphB2-/-; EphB3-/- mice, the boundary between the proliferative and differentiated cell compartments was not well defined and crypt proliferative cells intermingled with differentiated cells in the embryonic gut. Thus, EphB/ephrin-B signaling is necessary to establish cell compartments and to organize the ordered migration of epithelial cells along the crypt axis. EphB2/EphB3 double mutant mice also showed decreased proliferation in intestinal crypts [33]. It was postulated that this defect could be due to direct control of ISC proliferation by EphB signaling [33]. Yet, the recent finding that Paneth Cells secrete factors that promote ISCs maintenance [34] may indicate that the defects in Paneth cell compartmentalization present in EphB3 null mice may also indirectly affect ISC proliferation.

During embryonic development the role of EphB receptors in cell sorting, boundary formation and control of migration has been connected to their ability to generate repulsion between cell populations [30]. Given the fact that epithelial intestinal cells are intimately linked by a strong network of cell-to-cell contacts, it is unclear whether the lack of intestinal cell compartmentalization displayed by EphB or ephrin-B mutants can be solely explained by lack of repulsive signals. Computational modeling of crypt cell migration suggests that differential affinity

between the cell compartments along the crypt-villus axis (ISC, TA and differentiated) may be sufficient to set the ordered migration in the intestinal epithelium [35]. Evidence to support this prediction may come from two different observations. First, EphB signaling enforces E-cadherin-mediated aggregation and cell polarization in epithelial cells [32, 36]. Second, we have recently observed that this phenomenon is coupled to differential cell adhesion between EphB and ephrin-B expressing populations. EphB2 and EphB3 interact with E-cadherin and with the metalloproteinase ADAM10 [37]. Activation of EphB receptors induces the activity of ADAM10 at the interfaces with ephrin-B1 expressing cells. As a result, ADAM10 induces local cleavage of E-cadherin at the boundary between the two cell populations preventing adhesion amongst them. Transgenic mice engineered to express a dominant negative ADAM10 form in Paneth cells display abnormal cell positioning of this cell type, thus phenocopying EphB3 null mice [37]. Overall, these observations suggest a model by which EphB signaling would promote adhesion between cells localized at the crypt base. Yet, at the same time EphB-regulated ADAM10 activation would destroy E-cadherin-mediated adhesion between Paneth Cells (EphB3+) and those TA cells (ephrin-B1+) located immediately above. This mechanism would segregate Paneth cells from the upward migratory cell column and restrict their positioning to the crypt base.

EphB signaling and regulation of cell migration during CRC progression

Mutational activation of the Wnt pathway imposes a constitutive crypt progenitor phenotype at the onset of tumorigenesis which includes elevated expression of EphB2 and EphB3 receptors [28, 38]. We have recently explored the expression of EphB2 in late stage CRCs and noticed that in those tumors that retained a glandular structure the expression of EphB2 followed a pattern which was similar to that of the normal intestine [39]. Tumor cells marked by high levels of EphB2 expressed a gene program characteristic of intestinal stem cells including the expression of several ISC marker genes such as Lgr5 or Ascl2 [39]. On the contrary, tumor cells with low EphB2 surface levels expressed the transcriptional program of differentiated intestinal cells. Consistent with EphB2-high cells being

cancer stem cells, this population displayed long-term self-renewal potential and was capable of initiating tumors in immunodeficient mice with much higher efficiency than EphB2-low cells [39]. On the other hand, a few years ago ours and several other groups noticed that some CRCs silenced the expression of EphB2 and EphB3 as the tumor progresses. Most prominently, poorly differentiated CRCs as well as invasion fronts were EphB2 negative [38]. Indeed, EphB2 downregulation correlated with poor prognosis, cancer invasion and metastasis [40-41]. Genetic analysis in mouse models demonstrated that blockage of EphB activity in APCMin/+ background promoted the formation of aggressive colorectal tumors [32, 38]. Thus, EphB signaling blocks CRC progression. The mechanism responsible for EphB silencing in malignant colorectal tumors remains unknown, yet promoter methylation as well as point mutations in EphB2 have been found in a small subset of patients [42]. In addition, EphB2 expression is lost in hypoxic tumor areas [43]. But, how does EphB signaling block tumor progression and how this phenomena could be reconciled with EphB2 being a marker gene of cancer stem cells? Analysis of mouse models of CRC has revealed that at the onset of tumorigenesis, EphB+ tumor cells are incapable of invading the adjacent epithelium unless ephrin-B1+ is genetically deleted from these areas [32]. *In vitro*, activation of EphB2 or EphB3 receptors enforces growth into tight clusters of fully malignant cancer cells which would otherwise spread and unrestrictedly occupy the complete growth area [32]. This effect depends critically on the capacity of EphB signaling to enforce E-cadherin mediated adhesion on the EphB-expressing population [32]. Also, colorectal tumors derived from mice bearing EphB or ephrin-B1 mutant alleles fail to grow with glandular patterns and display downregulation of adherens and tight junctions components [32, 38]. Interestingly, independent works have also shown that re-expression of EphB3 reverts epithelial-to-mesenchymal transition in CRC cell lines [44] whereas in non-transformed breast cells blockade of endogenous EphB4 signaling induces an EMT-like phenotype [36]. Hence, EphB signaling may act as suppressor of EMT by promoting adhesion between epithelial tumor cells. This model is consistent with the observation that EphB2 silencing correlates with tumor budding in CRC, a histological feature characterized by small

nests of tumor cells that migrate away from the main tumor mass at invasion fronts [45]. Our current working hypothesis is that EphB2 marks the bulk cancer stem cell population which is organized into crypt-like niches [39] whereas EphB receptors are silenced in invading cancer stem cells to procure unrestricted dissemination.

Conclusions and future perspectives

In this review we have highlighted the alterations developed by mouse models carrying deletions of several adhesion molecules in the intestinal epithelium. Table I summarizes the phenotypes described along the text (Table I). Despite our wide knowledge of the mechanisms that regulate cell adhesion and migration in cell lines, *in vivo* studies are sparse. In our view, at least three central questions remain to be solved. First, the force that drives the migration of crypt cells is currently not well understood. The most widely accepted view is that migration of intestinal cells is a passive process that results from the continuous pressure imposed by rapidly dividing progenitor cells in the crypts which would push the existing epithelial monolayer upwards. Yet, it is also possible that additional mechanisms such as chemoattraction or repulsion participate in the regulation of migration. Second, we do not understand how the formation of a tight epithelial barrier is compatible with the plasticity required for constant cell renewal and migration. Proliferating progenitor cells in the crypts must constantly remodel cell adhesions to allow cell division which occurs by intercalation of newly formed cells into the epithelium. Furthermore, there is probably a need for rapid remodeling of the cell adhesion complexes when neighboring cells from any single crypt reach the surface epithelium and are segregated to populate two different adjacent villi. Third, we yet have to decipher the mechanism that allows ISCs to escape the upward migratory force. Our limited knowledge of these problems is in part derived from the impossibility of tracking the migration of intestinal cells *in vivo*. Recent developments such as the in vitro culture of intestinal organoids as well as advanced techniques of intravital microscopy will surely help answer these elusive questions.

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TABLE 1: Intestinal phenotypes of mice with genetic alterations in adhesion molecules.

Gene	Genetic manipulation	Intestinal phenotype	Ref.
E-cadherin	Conditional inducible intestinal KO (*Villin-CreER^{T2}*)	Hemorrhagic diarrhea, loss of intestinal architecture (including desmosomes), increased apoptosis, proliferation and migration, elongated crypts, defects in secretory cell maturation and localization (loss of Goblet cells, mispositioning of Paneth cells and presence of intermediate cells), defective Wnt-target gene expression at the ISC niche.	[1]
E-cadherin	Forced expression (*Fabp1*)	Slowed migration, decreased proliferation, increased apoptosis in the crypts.	[2]
N-cadherin	KI in one E-cadherin locus, KO of the other allele (*Villin-Cre*)	Death around weaning, increased proliferation and nuclear β-catenin, polyp formation, repressed BMP signaling, longer and sometimes branched villi, less frequency of differentiated cell types.	[3]
β-catenin	Conditional inducible intestinal KO (*Villin-CreER^{T2}; Ah-Cre*)	Lack of cell proliferation, increased apoptosis, detachment of epithelial sheet, crypt and ISC loss. No apparent defects in cell-cell adhesion.	[6-7]
p120-catenin	Conditional intestinal KO (*Villin-Cre*)	Death at P17 from hemorrhagic diarrhea and dehydration, loss of epithelial integrity and failure to form the intestinal barrier, increased proliferation and migration, increase in signals of inflammation and activation of RhoA effectors.	[9]
Ezrin	Full KO	Death at weaning, abnormal terminal web resulting in non-uniform microvilli and elongated apical junctions, round and fused villi.	[12]
Rac1 Constitutive Active (Leu61)	Forced expression (*Fabp1*)	Mortality at E18.5, precocious maturation of Paneth cells and enterocytes and incorrect apical β-actin localization.	[13]
Rac1 Dominant Negative (Asn17)	Forced expression (*Fabp1*)	Reduced cell migration, defects in secretory lineage differentiation, elongation of crypts, widening of villi.	[13]
Tiam-1	Full KO	Normal architecture, resistance to Wnt-induced adenoma formation but tumors present more aggressive behavior.	[15]
Claudin-15	Full KO	Megaintestine, increased length of proliferative compartment, increased size of villi, defects in paracellular Na+ permeability and glucose absorption.	[17-18]
Claudin-2	Full KO	Defects in paracellular permeability in infant intestine but not in adult intestine	[18]
N-cadherin DN	Forced expression (*Fabp1*)	Accelerated and disorganized migration, increased proliferation and apoptosis (throughout the entire crypt-villus axis), loss of cell-cell contacts, defects in differentiation. Inflammatory bowel disease.	[21]
HNF4A	Conditional intestinal KO (*Villin-Cre*)	Alteration of mucin-associated genes and increased intestinal permeability, susceptibility to acute colitis following an inflammatory insult.	[25]
HNF4A	Conditional intestinal KO (*Villin-Cre*)	Spontaneous inflammation in the colon	[26]

EphB2/B3	Full KO	Intermingling of proliferative and differentiated compartments, Paneth cell and ephrin-B expressing cell mispositioning. Reduced proliferation.	[28, 33]
EphB3	Full KO	Paneth cell mispositioning. Increased colorectal tumorigenesis in Apc^{min} background.	[28, 38]
Dominant Negative EphB2 ($EphB2^{\Delta Cyt}$)	Transgenic expression (*Villin*)	Mispositioning of ephrin-B expressing cells. Increased colorectal tumorigenesis in Apc^{min} background.	[28, 38]
Ephrin-B1	Conditional intestinal KO (*Villin-Cre*)	Mispositioned Paneth cells, lack of tumor cell compartmentalization through modulation of E-cadherin function by EphB/ephrin-B signaling.	[32]
EphB2 Constitutive Active (F620D) / EphB3	KI /KO	Enhanced progenitor cell proliferation.	[33]
EphB2 Kinase Inactive (β-gal) / EphB3	KI /KO	Reduced proliferation, Paneth cell mispositioning.	[33]
ADAM10 DN	Transgenic expression in Paneth cells (*Cryptdin2*)	Paneth cell mispositioning.	[37]

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