REVIEWS IN BASIC AND CLINICAL GASTROENTEROLOGY AND HEPATOLOGY

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Notch: Architect, Landscaper, and Guardian of the Intestine

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In the past decade, enormous progress has been made in understanding the role of stem cells in physiologic tissue renewal and in pathologic processes such as cancer. These findings have shed light on the identity and biological properties of such cells and the intrinsic and extrinsic signals that balance stem cell self-renewal with differentiation. With its astonishing self-renewal capacity, the intestinal epithelium has provided a unique model to study stem cell biology, lineage specification, and cancer. Here we review the role of Notch signaling in physiologic cell renewal and differentiation in the intestine as well as during its malignant transformation.

Keywords: Intestine; Stem Cells; Homeostasis; Notch; Colon Cancer.

The primary function of the intestinal tract is food digestion, the absorption of nutrients and water, and the cellular defense against pathogens and microorganisms. To withstand the demands of these functions, the epithelium lining of the intestinal tract has developed a remarkable capacity for cell renewal. Every day, thousands of cells are born and as many die in a continuous and highly regulated cycle of birth, differentiation, and death. In the past decade, the biological processes that underlie this astonishing capacity for self-renewal, lineage specification, and epithelial differentiation have been uncovered.[1] Defects in these biological processes disrupt normal intestinal homeostasis and architecture and underlie inflammatory disease and cancer.

Development, Cell Types, and Architecture of the Mammalian Intestine

During the early stages of embryonic development, endodermal cells undergo epithelial-to-mesenchymal transition, invaginate, and form the gut tube, which is gradually subdivided into 3 parts along the anterior-posterior axis: the foregut, midgut, and hindgut. The foregut will develop into the pharynx, esophagus, and stomach, while the midgut will give rise to the small intestine and the hindgut to large intestine (colon). Starting from approximately mouse embryonic day 14, through the upward movement of the underlying mesenchyme, the single-layered intestinal epithelium forms finger-like projections into the gut lumen (the villi). After birth, crypts form between villi by invagination into the underlying connective tissue. Reciprocal signaling between the epithelium and the mesenchyme shapes intestinal morphogenesis.[2]

Three weeks after birth, development of the adult gut is complete and can be divided into the small and the large intestine. Along the rostral-caudal axis, the small intestine can be subdivided into duodenum, jejunum, and ileum. The functional unit of the small intestine is a villus containing terminally differentiated cells connected to the crypts of Lieberkühn, which harbor the proliferative compartment. The colon is only composed of crypts and has no extending villi. The intestine is a highly dynamic organ system with a turnover rate of approximately 60 hours for the entire epithelial population.[3] The proliferating cells in the crypt are intestinal stem cells (ISCs) and transit amplifying (TA) cells that fuel the continuous production of several differentiated epithelial cell types (Figure 1). The crypt epithelium cycles asynchronously, and new crypts arise though bifurcation (crypt fission) during adult life as the intestinal tract continues to grow.[4] Within a week, progenitors migrate upward from the crypt into the villus tip, from which they are shed into the intestinal lumen. To support this high turnover, mouse crypt stem cells are estimated to undergo a thousand divisions during their lifetime. Whereas stem cell self-renewal occurs within the crypt throughout adult life, TA daughters continue to differentiate to produce 2 main types of differentiated cells: the absorptive cells or enterocytes (ECs) and the secretory cells. The ECs are involved in nutrient uptake

Abbreviations used in this paper: AES, Amino terminal Enhancer of Split; AMP, adult midgut progenitor; bHLH, basic helix-loop-helix; CBC, columnar base cell; Dll, Delta-like ligand; EB, enteroblast; EC, enterocyte; EE, enteroendocrine; GSI, γ-secretase inhibitor; Hes, Hairy Enhancer of Split; ISC, intestinal stem cell; KLF4, Krüppel-like factor 4; Mib1, Mindbomb-1; NICD, Notch intracellular domain; PC, peripheral cell; Pofut, O-fucosyl transferase1; SPDEF, SAM pointed domain ETS transcription factor; Su(H), Suppressor of Hairless; TA, transit amplifying.

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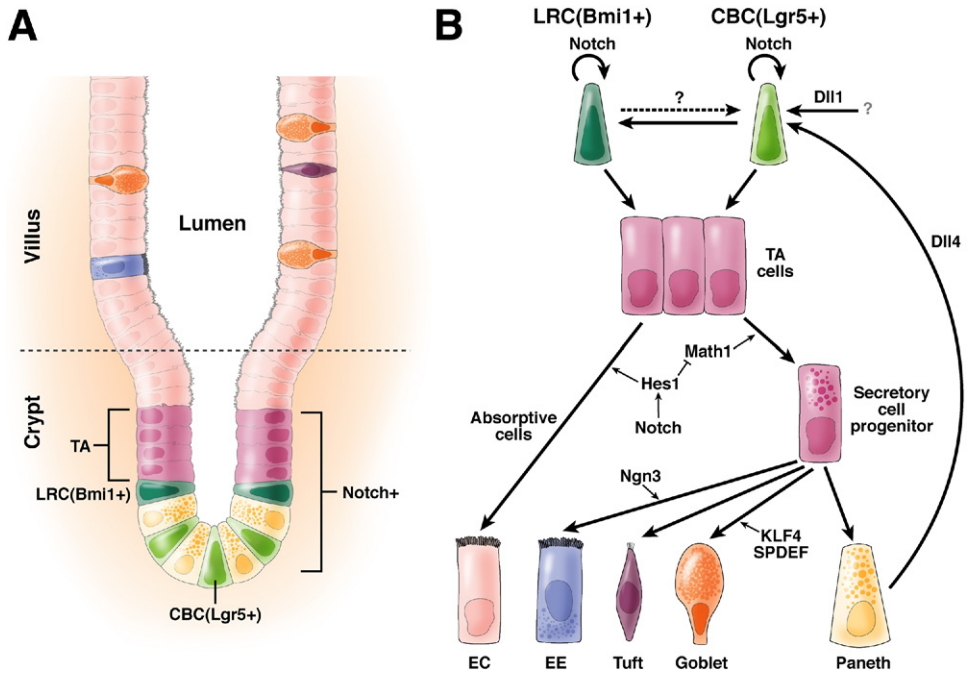

Figure 1. The mammalian small intestine and role of Notch in ISC renewal. (*A*) Two populations of stem cells, CBCs and label retaining cells (LRCs), characterized by Lgr5 and Bmi1 expression, respectively, are located at the bottom of the crypt. Their daughters, the TA cells, undergo rapid proliferation as they move upwards. Terminal cell differentiation starts at about the upper third of the crypt and gives rise to 5 major cell types: the absorptive ECs and the secretory cell types composed of EE cells, tuft cells, goblet cells, and Paneth cells. Whereas most differentiated cells move and home into the villi, Paneth cells move back into the bottom of the crypt after their birth to form the niche to orchestrate ISC renewal and differentiation. (*B*) The relationship between the LRC and CBC stem cell populations is not very clear. It is possible that they could give rise to each other (*dashed arrows*). Although both populations produce TA cells, it has been proposed that CBCs may represent a rapidly cycling population (*thick arrow*) whereas LRC is a slow cycling one (*thin arrow*). Notch may be required for the maintenance of both. Although Paneth cells express Dll4 ligand, it remains unclear which cells provide the Dll1 ligands, which play a dominant role in sustaining stem cell self-renewal as well. During cell fate specification, Notch promotes the EC fate and inhibits the secretory cell fates through down-regulation of Math1, a master transcription factor for all secretory cell fates. Math1 controls secretory commitment by activation of cell type-specific transcription factors. For example, Ngn3 specifies the EE cell fates while KLF4 and SPDEF goblet cells.

and secretion of hydrolases and constitute the majority of cells in the villus epithelium. Secretory cells consist of the mucus-producing goblet cells, the hormone-producing enteroendocrine (EE) cells, and the lysozyme-producing Paneth cells. Paneth cells migrate downward into the crypt base, where they live for several weeks. Recently, tuft cells were recognized as a fourth kind of secretory cell, which secrets opioids and cyclooxygenase enzymes.[5]

Evidence for the Existence of ISCs

Clonal analysis performed several decades ago showed that all differentiated cell types arise from a few stem cells residing in the monoclonal crypts and contribute a column to the polyclonal villi.[6–9] Early lineage tracing studies revealed a slow proliferating or quiescent stem cell located about 4 cells above Paneth cells (at the +4 position).[10] Lineage tracing studies based on stem cell markers have delineated the location of 2 distinct ISC pools in the crypt[11,12]: (1) the columnar base cells (CBCs)[13] with a high turnover, expressing the leucine-rich repeat membrane protein Lgr5,[11] and (2) a quiescent label-retaining population located 4 cells above the Paneth cells, expressing the polycomb protein Bmi1.[14,15] A model emerging from these findings is that 2 distinct stem cell populations ([Lgr5+, CBC] and [Bmi1, +4]) act cooperatively to support normal physiologic cell replenishment and tissue repair.[16] However, +4 cells are also labeled with Lgr5 lineage markers,[12] suggesting that both lineages are derived from CBCs. Coordination between cell renewal, transit amplification, terminal differentiation, and apoptosis requires a precise interplay among several signaling pathways, which is invariably perturbed during intestinal diseases such as cancer. In this review, we will focus on the role of the Notch signaling pathway, a master regulator of cell fate during intestinal homeostasis. We summarize the expression patterns and functions of the core components of the pathway, starting at the membrane with the Notch ligands and receptors and ending with target gene activation in the nucleus. Genetic and chemical gain- and loss-of-function studies are also discussed to illustrate the important roles of the Notch pathway in intestinal homeostasis.

The Core Components of the Notch Signaling Pathway

The Notch signaling pathway is a highly conserved short-range communication mechanism used in all metazoans.[17,18] Notch genes encode large type I transmem-