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REVIEWS IN BASIC AND CLINICAL GASTROENTEROLOGY AND HEPATOLOGY

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Stem Cells Marked by the R-Spondin Receptor LGR5

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Since the discovery of LGR5 as a marker of intestinal stem cells, the field has developed explosively and led to many new avenues of research. The inner workings of the intestinal crypt stem cell niche are now well understood. The study of stem cell–enriched genes has uncovered some previously unknown aspects of the Wnt signaling pathway, the major driver of crypt dynamics. LGR5$^+$ stem cells can now be cultured over long periods in vitro as epithelial organoids or "mini-guts." This technology opens new possibilities of using cultured adult stem cells for drug development, disease modeling, gene therapy, and regenerative medicine. This review describes the rediscovery of crypt base columnar cells as LGR5$^+$ adult stem cells and summarizes subsequent progress, promises, unresolved issues, and challenges of the field.

Keywords: Stem Cell; LGR5; Organoid; Intestine.

The intestinal epithelium renews every 3 to 5 days in adults. This rapid turnover is sustained by tissue-specific stem cells located in crypts. Original studies searching for the identity of these cells applied electron microscopy, DNA label retention, and staining with candidate markers for rare cells.[1–5] Cheng and Leblond in particular identified a slender cell type, squeezed in between the Paneth cells at crypt bottoms, and proposed that these crypt base columnar cells represent the crypt stem cells.[2] Independently, DNA label-retaining cells were reported by Potten et al to reside around "position 4" of the crypts.[6,7] Several markers of the +4 cells have been proposed, including BMI1,[8] TERT,[9] HOPX,[10] and LRIG1.[11] Their specificity as +4 markers has been challenged by subsequent expression analysis with single-molecule in situ hybridization.[12,13] A recent study by Buczacki et al[14] reconciled the crypt base columnar and +4 stem cell models by showing that quiescent cells at the +4 position are committed secretory precursors that can revert to stem cells on tissue damage.[15] The focus of this review is on adult stem cells marked by LGR5.

The advent of genetic lineage tracing using Cre recombinase allowed the unequivocal confirmation of crypt base columnar cells as long-lived, multipotent crypt stem cells. In 2007, Barker et al generated a lineage tracing mouse by inserting green fluorescent protein (GFP) and the tamoxifen-inducible CreERT2 fusion protein into the *Lgr5* locus, a Wnt target gene exquisitely expressed in crypt base columnar cells.[16] These cells divide every 24 hours, generating rapidly proliferative progenitors that fill the pocket-like crypts (Figure 1*A*). Proliferating progenitors migrate upward while they differentiate into nutrient-absorbing enterocytes and secretory cells that produce mucins (goblet cells, Figure 1*B*, *orange arrows*) or hormones (enteroendocrine cells, Figure 1*B*, *purple arrows*). These 3 cell types make up the epithelium of the villus, a digit-like protrusion into the gut lumen. Paneth cells also belong to the secretory lineage, but they migrate downward to reside together with crypt base columnar cells (Figure 1*A* and *B*, *yellow arrow*). They secrete antibacterial components and create a home for stem cells by providing signals (eg, EGF, NOTCH, and WNT ligands) important for stem cell maintenance.[17] Two cell types, tuft cells (Figure 1*B*, *violet arrows*) and M cells (Figure 1*B*, *dark orange arrows*), are rarely forming descendants of LGR5$^+$ stem cells.[18,19] Tuft cells possibly serve a sensory role for luminal contents, whereas M cells, restricted to the epithelium overlying Peyer patches, capture and transport antigens to immune cells in the patches (Figure 1*A*).

This review highlights the confirmation of crypt base columnar cells as LGR5$^+$ intestinal stem cells and provides an in-depth overview of subsequent discoveries made on these cells. As an unexpected offshoot, the LGR5 knock-in mice were instrumental in identifying several additional adult stem cells in other tissues (eg, stomach pylorus[20] and colonic[16] epithelia, hair follicles,[21] mammary glands,[22,23] kidney,[24] and epithelia of inner ear[25,26] and ovarian surface[27]). With the LGR5 knock-in models, unexpected dynamics of adult stem cells at the stem cell zone were revealed,[28,29] while plasticity of committed progenitors in terms of their ability to revert to a stem cell phenotype were described.[14,30,31] Genetic studies of intestinal stem cell genes have uncovered the hidden nature of stem cell regulation and maintenance.[32–35] Finally, the cumulative understanding of the intestinal stem cells facilitated the development of a genetically and phenotypically stable primary 3-dimensional culture system, so-called intestinal

Abbreviations used in this paper: **ER, estrogen receptor; GFP, green fluorescent protein; NF-κB, nuclear factor κB; OLFM4, Olfactomedin-4; RSPO, R-spondin.**

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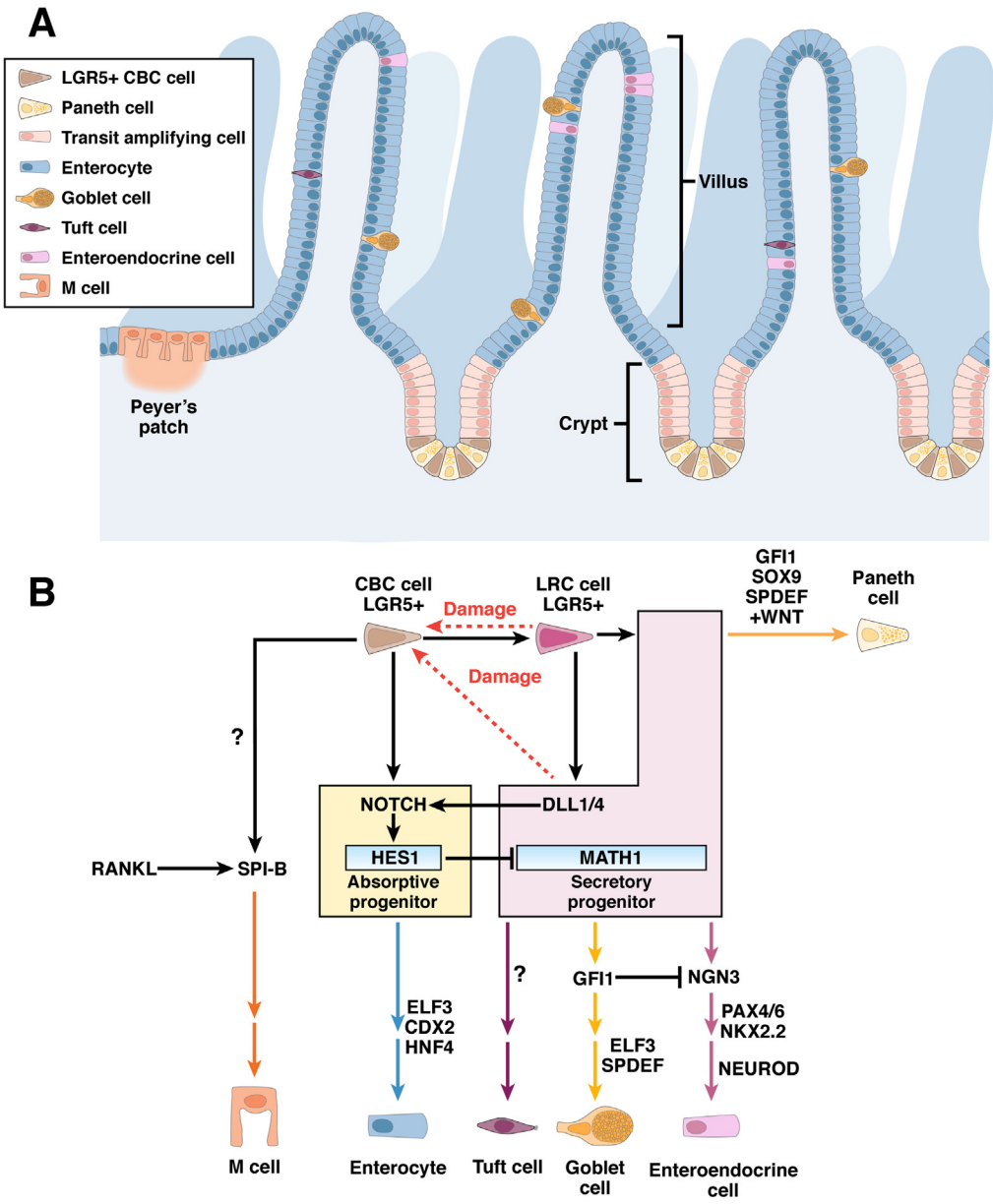

Figure 1. (*A*) Structure of the small intestinal epithelium. The inner surface epithelium of the small intestine is folded into villi, which resemble digit-like protrusions, and crypts corresponding to pocket-like structures. The LGR5$^+$ crypt base columnar stem cells, located at the bottom of the crypt, are interspersed between Paneth cells, which constitute the niche cells. Through daily proliferation, the stem cells give rise to progeny called transit-amplifying cells. During their upward migration, the progeny differentiate into nutrient-absorbing enterocytes as well as other secretory cells that produce mucins (goblet cells) and hormones (enteroendocrine cells). M cells and tuft cells are rarer cell types present in the small intestine. (*B*) Lineage specification. Six differentiated cell types populate the small intestine. Enteroendocrine, goblet, and Paneth cells belong to the secretory lineage, whereas enterocytes originate from the absorptive lineage. Enterocytes and goblet cells are most abundant, whereas enteroendocrine, M, and tuft cells are less common. LRC, label-retaining cell; CBC cell, crypt base columnar cell; M cell, microfold cell.

organoids.[36] The key was the growth factor cocktail that supports unlimited propagation of adult stem cells in vitro. Later, this basic cocktail was modified to grow a number of LGR5$^+$ progenitor-based organoids from different tissues.[20,37–39] Using this novel culture system, in vitro genetics,[40] in vitro disease modeling,[41,42] and targeted gene repair in adult stem cells[43] have become possible.

Identification of LGR5$^+$ Intestinal Epithelial Stem Cells

Following Ahn and Joyner's lineage tracing experiment from subventricular zone stem cells in the brain using *Gli1-CreERT2;Rosa26-reporter* mice,[44] lineage tracing experiments have become the gold standard for investigating