Lrig1: a new master regulator of epithelial stem cells

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The intestine represents the most vigorously renewing, adult epithelial tissue that makes maintenance of its homeostasis a delicate balance between proliferation, cell cycle arrest, migration, differentiation, and cell death. These processes are precisely controlled by a network of developmental signalling cascades, which include Wnt, Notch, BMP/TGF β , and Hedgehog pathways. A new, elegant study by Wong *et al* (2012) now adds Lrig1 as a key player in the control of intestinal homeostasis. As for epidermal stem cells, Lrig1 limits the size of the intestinal progenitor compartment by dampening EGF/ ErbB-triggered stem cell expansion.

The epithelium of the small intestine is separated into two distinct compartments: a proliferative crypt, containing tissue-specific stem cells, and a villus with differentiated, short-lived cells, which are replenished by a constant stream of cell migration from the underlying crypt (Scoville *et al*, 2008). In particular, the canonical Wnt pathway in combination with Notch signals control stem cell maintenance and proliferation in the crypt. In addition, both pathways direct differentiation into the Paneth and the absorptive cell lineage, respectively. Intensive cross-talk between the epithelium and the underlying mesenchyme helps to define the crypt–villus boundary. This relies on epithelial-derived Hedgehog and Wnt ligands that trigger stromal BMP production, which in turn signals back to the epithelium to restrict proliferation to the crypt. A gradient of BMP antagonists produced by mesenchymal cells at the bottom of the crypts supports compartmentalization. In addition, a Wnt gradient in the crypt defines EphB expression and establishes repulsion-mediated separation into Paneth

cell, proliferative, and differentiation zones along the crypt–villus axis (Figure 1A).

In the small intestine, two stem cell (SC) populations coexist: Lgr5⁺ crypt base columnar cells (CBCs) that cycle every 24 h and are interspersed between Paneth cells, and slower dividing SCs concentrated above (around position +4 relative to the crypt bottom) the Lgr5⁺ position (Takeda *et al*, 2011). The localization of these Hopx⁺ mTert⁺ slowly cycling SCs partly overlaps with that of quiescent cells, which show long-term label retention upon irradiation damage and pulse labelling with BrdU. Lgr5⁺ CBCs are, however, dispensable (Tian *et al*, 2008) and can be replaced by the second stem cell population, which also shows greater activity during damage repair. The relationship between these two stem cell populations, which can reciprocally generate each other, and the mechanisms that govern quiescence are being elucidated. Importantly, leucine-rich repeats and Ig-like domains 1 (Lrig1), a transmembrane protein that interacts with ErbBs and promotes its degradation, has now been found to be enriched at the crypt base and in the progenitor compartment of the small intestine and colon (Wong *et al*, 2012). Lrig1 is highly expressed in Lgr5⁺, Musashi1⁺, Ascl2⁺, and Olfm4⁺ CBCs, and shows an inverse relation to the pattern of activated, phosphorylated EGFR above the crypt base (Figure 1A). In line with these patterns, deletion of Lrig1 in the mouse causes a dramatic crypt expansion and increased numbers of CBCs, transit-amplifying and Paneth cells. Whether the increase of Paneth cells, which actually do not express Lrig1, is a secondary effect due to the progenitor expansion remains open. Importantly, reduction of EGFR

Figure 1 (A) The epithelium of the small intestine contains two populations of multipotent stem cells that reside at the bottom of the crypts. These give rise to transit-amplifying progenitors, which rapidly divide while migrating upwards. Cell cycle arrest and functional differentiation occur when these cells pass from the upper part of the crypt into the villus where they continue their upward movement until they finally undergo apoptosis. Only long-living Paneth cells follow a different path as they migrate downwards to populate the base of the crypt. Control of proliferation and lineage specification of all intestinal epithelial cells is directed in a self-organizing, dynamically regulated process based on cell–cell and cell–environment interactions. Among them, Wnt and Notch signalling have been defined as major determinants for stem cell maintenance, for proliferation of stem cells in the crypt and lineage specification. Epithelial-derived Hedgehog ligands and reciprocal stromal BMP ligands establish a connection between the epithelium and the stroma that regulates the crypt–villus boundary. In addition, repulsive interactions mediated by the Eph/ephrin family allow establishment of stable compartments. Importantly, ErbB signalling, which is partially suppressed by Lrig1 at the base of the crypt, is now shown to be a new key player in the control of stem and progenitor cell expansion. (B) Cross-talk of signalling pathways in intestinal homeostasis with an emphasis on ErbB signalling. A negative feedback loop via Lrig1 helps to fine-tune population size and proliferative activity of intestinal progenitor cells. Lrig1 has been identified as a direct target of Myc and is known to repress ErbB signalling. Myc itself is a main target of the ErbB and Wnt pathways implicated in intestinal stem and progenitor cell expansion. Moreover, Lrig1 has been found to promote BMP signalling, which interferes with intestinal proliferation by restricting AKT activation via PTEN.

signalling by pharmacological (Gefitinib) and genetic modulation (Egfr^{wa-2} mice) is able to partially normalize all Lrig1 phenotypes. These data establish EGF/ErbB signalling, as an important regulator of the crypt compartment, and suggest Lrig1 as a central control that dampens the expansion of stem cells during normal intestinal homeostasis.

Lrig1 was initially identified in the skin and proposed to maintain epidermal stem cells in a quiescent state (Watt and Jensen, 2009). Lrig1 marks human interfollicular epidermal stem cells, which can give rise to all epithelial lineages including hair follicle cells in skin reconstitution assays. However, during normal homeostasis, these cells are only bipotent, contributing to the sebaceous gland and the interfollicular epidermis. In contrast to quiescent Lrig1⁺SCs in the skin, Lrig1⁺ intestinal SCs are rapidly dividing and Lrig1 appears to only reduce their proliferative capacity. However, similar to the situation in the skin, Lrig1 and EGF signalling may play an important role during damage repair. Earlier experiments analysed the phenotype of mice lacking major EGF family members (Egger *et al*, 1997; Troyer *et al*, 2001). While these mice display some duodenal lesions during normal homeostasis, further experiments established EGF signalling as a key protective component that ameliorates mucosal damage. It remains to be seen whether activation of intestinal SCs during damage repair involves mitigation of Lrig1 dampening.

Lrig1 is known to repress ErbB signalling by mediating ubiquitinylation and degradation of activated receptors, thereby limiting the amplitude of EGF signalling (Watt and Jensen, 2009). Consequently, Lrig1 deletion in the intestine induced upregulation of EGFR, ErbB2, and ErbB3, promoting downstream activation of c-Myc within intestinal stem and progenitor cells (Wong *et al*, 2012). Importantly, *Lrig1* is a direct Myc target gene, and thereby part of a negative feedback loop that helps to fine-tune the population size

and proliferative activity of intestinal progenitor cells (Figure 1B).

Since the rescue of the Lrig1^{-/-} phenotype by EGFR deficiency was only partial (Wong *et al*, 2012), other mechanisms may contribute. Intriguingly, Lrig1 has been shown to promote BMP signalling by direct binding to Type I (ALK6) and Type II (ALK1, ALK2, ALK3, and ActRIB) BMP receptors (Gumienny *et al*, 2010). BMPRIa inactivation, deficiency of its downstream effector PTEN, and transgenic overexpression of the BMP inhibitor Noggin display crypt expansion and increased SC numbers. Inhibition of BMP signalling in these genetic models enhanced AKT activation and increased Wnt signalling, promoting proliferation and adenoma formation (Figure 1B; Scoville *et al*, 2008). Future work will reveal a potential involvement of BMP and Wnt signalling in the Lrig1 knockout phenotype.

The ErbB pathway has been linked to inflammatory bowel disease, and progression and metastatic potential of colorectal cancer. EGFR inhibition blocks adenoma formation in preclinical models, and ErbB pathway inhibition is currently being evaluated in clinical trials with colorectal cancer patients, where promising results have been reported (Cunningham *et al*, 2004). In contrast, Lrig1 is expressed at low levels in several cancer types but is overexpressed in some prostate and colorectal tumours (Hedman and Henriksson, 2007). Given this heterogeneity, the Lrig1 function in tumours appears to be cell- and context-dependent. Due to early postnatal lethality of Lrig1 knockout mice, the exciting possibility that Lrig1 may act as an intestinal tumour suppressor could not be answered by the current study but clearly deserves further attention.

Conflict of interest

The authors declare that they have no conflict of interest.

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