The nature of intestinal stem cells' nurture

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The 'nature versus nurture' debate concerns the relative contributions to an individual's identity of its nature (that is, its genetic make-up) compared with its nurture, defined as the totality of external, environmental factors. A similar type of debate is ongoing among developmental and stem-cell biologists: is the intrinsic nature (that is, its (epi)genetic make-up) of a stem cell what makes it self-renew and differentiate according to the physiological needs of a given tissue, or is it the immediate environment (nurture) that regulates stemness? Irrespective of the relative weight of each contribution, there is little doubt that both cell-autonomous and environmental factors play crucial roles in the maintenance of homeostasis in self-renewing tissues such as the skin, mammary gland, blood and intestine. In an article published last month in *EMBO reports* (Mustata *et al*, 2011), the *Lgr4* gene is shown to have a rate-limiting role in establishing the stem-cell niche of the proximal intestinal tract.

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The epithelial lining of the proximal intestine is characterized by a unique tissue architecture consisting of villi and crypts. The intestinal crypt of Lieberkühn is a highly dynamic niche with stem cells in its lower third, which give rise to a population of fast-cycling transit-amplifying cells. Transit-amplifying cells undergo a limited number of cell divisions and eventually differentiate into four specialized cell types of the small intestine: absorptive, enteroendocrine, goblet and Paneth cells. Notably, Paneth cells are the only terminally differentiated cell type of the proximal intestinal tract that (i) move downwards along the crypt-villus axis and (ii) retain canonical

Wnt signalling activity upon differentiation (van Es *et al*, 2005).

On the basis of clonal analysis and knock-in experiments, it was shown that the crypt base columnar (CBC) cells—located in the lower third of the crypt and characterized by *Lgr5* expression—represent actively cycling stem cells that are able to give rise to all differentiated cell types of the intestinal epithelium (Barker *et al*, 2007). More recently, it has also been shown that Paneth cells, apart from their well-known bactericidal function, are in close physical association with *Lgr5*⁺ stem cells, to which they provide essential niche signals such as EGF, Wnt3a and Dll4 (Sato *et al*, 2011). This is also important in the light of the observation that single *Lgr5*⁺ stem cells, when cultured *ex vivo*, can generate crypt-villus organoids without a (mesenchymal) niche (Sato *et al*, 2009). In fact, the latter is only partly true, as these organoids are cultured in matrigel and in the presence of specific growth factors that are probably released by the niche *in vivo*.

Lgr5, together with *Lgr4* and *Lgr6*, belongs to the family of leucine-rich repeat-containing G-protein-coupled seven-transmembrane receptors. Recently, both *Lgr5* and *Lgr6* have received attention from the stem-cell community: *Lgr5* is a downstream Wnt target gene and a marker of cycling stem cells in the intestinal tract and the hair follicle, whereas *Lgr6* expression marks adult stem cells in the skin (Barker & Clevers, 2010). However, whether they merely represent stem-cell markers or also have a functional role in stemness is unknown.

Loss of *Lgr4* function results in [...] an 80% reduction in Paneth-cell differentiation

Mustata *et al* (2011) report on the functional role of another member of the *Lgr* family, *Lgr4*, by studying the effects of a

targeted loss-of-function mutation (*Lgr4* KO) on the development and differentiation of the mouse small intestine both *in vivo* and *ex vivo*. Endogenous *Lgr4* expression is detected in transit-amplifying cells above the Paneth-cell zone, in CBC cells, and in rare Paneth cells. Loss of *Lgr4* function results in a reduction in crypt depth due to a 50% decrease in epithelial-cell proliferation and, surprisingly, in an 80% reduction in Paneth-cell differentiation. Strikingly, these phenotypic features are apparently antagonistic to those of *Lgr5* KO mice, in which premature Paneth-cell development was observed (Garcia *et al*, 2009). Accordingly, loss of *Lgr4* function partly rescues the perinatal lethality of *Lgr5* KO mice indicating non-redundancy of their individual functions.

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To further investigate the role of *Lgr4* in crypt development, the *ex vivo* 'minigut' culture system (Sato *et al*, 2009) was used; in contrast to crypts from wild-type mice that give rise to self-renewing structures encompassing all the differentiated cell lineages of the adult gut, organoids derived from age-matched *Lgr4* KO animals are initially present as hollow spheres, mainly composed of stem and transit-amplifying cells, which disaggregate within 2–3 days and die within a week in culture. In agreement with their apparently opposite and non-redundant functions, crypt cultures from *Lgr5* KO mice survive long-term culture and develop into differentiated organoids comparable with those of normal mice. Whereas loss of *Lgr4* function partly rescues the lethality of *Lgr5* KO mice *in vivo*, this is not true *ex vivo*; compound homozygous

Fig 1 | Schematic illustration of the intestinal stem-cell compartment in the upper intestinal tract: Lgr4 (expressed in CBC and TA cells) positively stimulates Paneth-cell differentiation and, indirectly, stem-cell homeostasis, while Lgr5 (expressed in CBC cells) has been reported to inhibit Paneth-cell differentiation (Garcia *et al*, 2009). CBC, crypt base columnar; Dll4, delta-like 4; EGF, epidermal growth factor; TA, transit amplifying.

Lgr4/5 KO crypts give rise to hollow spheres that collapse and die as observed in *Lgr4* KO organoids. Hence, under these experimental conditions—that is, in the absence of a mesenchymal niche—the *Lgr4* defect is dominant over the *Lgr5* one.

Analysis of Paneth-cell differentiation markers and of Wnt targets, including *Lgr5*, confirmed their downregulation in *Lgr4* KO organoids, thus suggesting a role for Lgr4 in Wnt signalling. Notably, lithium chloride treatment partly rescues the *ex vivo* phenotype of *Lgr4* KO crypts, although this is not the case for other Wnt-signalling agonists, such as *Wnt3a* and *Gsk3β* inhibitors. On the basis of these observations, the authors conclude that Lgr4 probably has a permissive, rather than a direct and active role in Wnt signalling.

In view of this and other studies, a revisitation of the cell-autonomous and niche-independent features of the *Lgr5*⁺ cycling

stem cell (CBC cells) in the intestinal crypt seems to be necessary (Fig 1). First, the capacity of CBC cells to recapitulate *ex vivo* the complexity of the crypt–villus unit is mostly dependent on Paneth cells (Sato *et al*, 2011). When they are sorted as single cells, CBC cells perform poorly in organoid formation, whereas doublets of CBC and Paneth cells show high clonogenicity (Sato *et al*, 2009, 2011). However, rather than occurring exclusively through the secretion of niche signals in the form of Wnt ligands, the nature of the interdependency between Paneth cells and CBC cells seems to involve additional mechanisms. As shown by Mustata *et al*, loss of *Lgr4* function causes a Paneth-cell differentiation blockade in the presence of wild-type levels of *Wnt3a* and *Wnt11*, a defect that can be rescued by lithium chloride, but not by the *Wnt3a* ligand or *Gsk3β* inhibitors. This indicates that additional factors secreted by epithelial and possibly mesenchymal

cells—for example, stromal myofibroblasts (Vermeulen *et al*, 2010)—and the physical association of Paneth with *Lgr5*⁺ cells underlies their ‘partnership’ in preserving homeostasis within such a highly dynamic tissue. Hence, Paneth cells apparently constitute an essential component of the stem-cell niche in the upper intestinal tract.

As it is always the case, good science leads to new questions. Which cell type provides this niche function in the colon where Paneth cells are not present? Of note, it has been shown that in the colon *Lgr5*⁺ cells are intermingled with yet uncharacterized CD24⁺ cells (Sato *et al*, 2011), a cell-surface antigen known to enrich for Paneth cells in the upper intestinal tract. As CD24 expression does not mark CBC cells, but rather their flanking cells, these observations could again reflect the supportive, niche role of Paneth cells and CD24⁺ cells in the upper and distal intestinal tract, respectively. This might also be true for colon cancer, where Paneth cells are often present, possibly to provide niche support for cancer stem cells. Alternatively, premature (in the colon) and/or fully differentiated (in the upper intestine) Paneth cells might have a dual function by providing physical and paracrine support for cycling stem cells in homeostasis, as well as representing the hitherto elusive quiescent stem cells that underlie tissue regeneration after tissue insults. Whatever the truth, the intestinal scene is now set to further dissect the complexity of the nature–nurture interaction between intestinal (cancer) stem cells and their niche.

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