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Epithelial and mesenchymal contribution to the niche: a safeguard for intestinal stem cell homeostasis

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The intestinal stem cell (ISC) niche is a highly complex microenvironment that supports stem cell maintenance and precisely orchestrates the balance between proliferation and differentiation. Here, multiple signaling molecules emanating from both the underlying mesenchyme and crypt-based epithelial cells cooperate to govern stem cell homeostasis (Figure 1). Understanding the cellular and molecular constituents of the niche is of critical importance to harness stem cells for tissue regeneration and to elucidate disease mechanisms. The conserved Wnt, Notch and epidermal growth factor (EGF) signaling pathways are identified regulators of stem cell behavior, however resolution of the cellular players and the impact of signaling molecules on different progenitor populations residing within the niche remain poorly understood. In this issue of *Gastroenterology*, a group led by Hans Clevers (Farin et al.)^[1] specifically address the niche requirement of Paneth cell (PC)-derived *Wnt3* in mediating intestinal homeostasis. Analysis of *Wnt3*-null epithelia in both *in vivo*, and *in vitro* cultures resulted in no apparent effect within the intact small intestine, but lack of growth in the culture system. While PCs have been defined to express *Wnt6* and *9b* *in vivo*, PCs did not express adequate levels to rescue the phenotype *in vitro*; instead, addition of the mesenchymally-expressed *Wnt2b* ligand supported enteroid growth. These findings highlight the robust compensatory signaling support provided by the underlying mesenchymal niche cells despite loss of an epithelial Wnt source.

The stem cell niche regulates intestinal stem cell behavior

The physical invagination of the intestinal crypt provides a functional ISC niche that consists of mesenchymal and epithelial cells within intimate proximity to facilitate tight regulation of the epithelial stem and progenitor populations^[2]. Cells within the mesenchyme cluster around the base of crypts and include mesenchymal stem cells, sub-epithelial myofibroblasts, smooth muscle cells of the muscularis mucosae, lymph and vascular endothelial cells, and a variety of bone-marrow derived stromal cells^[3]. This microenvironment contains additional factors including nerve cells and extracellular matrix components^[4]. The cell census and actual contribution of each cell type in regulating ISCs are not clear. Epithelial niche constituents include both differentiated and progenitor cells, anchored at or near the crypt-base via cellular adhesions. Finely tuned positive and negative feed-back signaling between niche cells regulates stem cell behavior during tissue homeostasis and regeneration^[2].

It is believed that the intestinal epithelium is maintained by two distinct pools of ISCs. The first are the quiescent ISCs located at the +4 position^[5], more recently defined by their expression of *Bmi1*, *Hopx*, *mTert* and *Lrig1*^[6-9], and the second are the rapidly cycling crypt-based columnar cells (CBCs) expressing the Wnt target gene *Lgr5*^[10]. While the

hierarchical relationships between these two ISC pools are only beginning to be uncovered^[9, 11, 12], it is evident that each population has different signaling requirements to govern their behavior during tissue homeostasis and regeneration^[5]. Lineage tracing experiments verify that cells expressing each of these markers are multipotent stem cells capable of giving rise to the major differentiated epithelial lineages: absorptive enterocytes and secretory enteroendocrine, tuft, goblet and PCs. Unlike other differentiated lineages, PCs are the only one to reside in the crypt-base, directly below quiescent +4 ISCs and interspersed among cycling *Lgr5*⁺ CBCs. PCs have a well-established immune role; producing antimicrobial agents that protect the epithelium from bacterial pathogens^[13]. Recent evidence supports additional niche functions for PCs including production of important signaling factors--canonical Wnt ligands *Wnt3*, *Wnt6* and *Wnt9b*, Notch ligands *Dll-1* and *Dll-4*, and *EGF*^[14, 15]-- but their essential role within the ISC niche is unclear.

Paneth cells are a source of signaling ligands

The Wnt signaling pathway is the best characterized regulator of intestinal homeostasis^[16]. In the context of canonical Wnt signaling, Wnt receptors internalize secreted Wnt ligands, triggering β -catenin stabilization, nuclear translocation and subsequent transcriptional activation of target genes involved in cell survival and proliferation. While over-activation of Wnt in the intestine is tumorigenic^[17], inhibiting Wnt causes loss of proliferative crypts and tissue attrition^[18], emphasizing a requirement for its precise modulation. Canonical Wnt signaling is most active in the crypt-base where CBCs and PCs reside. In CBCs, Wnt signaling regulates proliferative capacity, while PC Wnt-target expression influences homing and maturation^[19, 20]. Farin et al. further describe a role of Wnt-mediated PC maturation by elegantly demonstrating that forced *Wnt3* expression drives progenitors toward a PC fate, thus defining a signaling loop where PCs produce the Wnt ligands that are required for their maturation^[1].

PC production of signaling ligands substantiates their regulatory role within the stem cell niche specifically by communicating with the adjacent *Lgr5*⁺ CBCs. Despite this, requirement of PC-derived factors within the niche is unclear. Diphtheria toxin-mediated PC ablation does not overtly affect epithelial proliferation nor lineage allocation^[21]. However, re-analysis of this model demonstrates incomplete PC loss and yet a concomitant decrease in CBC numbers^[15]. While these studies did not definitively specify a niche role for the PC, they provided further support that an intricate relationship exists between Paneth and CBCs populations. The fact that PCs are physically juxtaposed to +4 ISCs presents an intriguing possibility that they may also participate in regulating this population.

Additionally, the importance of CBC and PC communication is evident from *in vitro* intestinal enteroid^[22] cultures. In the absence of mesenchymal cells, single *Lgr5*-expressing CBCs can form intestinal enteroids at low efficiency, generating structures harboring proliferative crypt-like and differentiated domains similar to native tissue architecture^[23]. Enteroids contain all differentiated epithelial lineages present in the intestine, including conserved PC-*Lgr5*⁺ CBC spatial relationships. Not surprisingly, recent studies show that ISCs co-cultured with PCs or the addition of exogenous *Wnt3a* to culture medium vastly improves growth efficiency of *Lgr5*⁺ cells^[15]. While these observations reinforce that PCs are integral niche components, recent data^[24, 25] has called into question the niche function of PCs.

Two recent studies showed that deletion of *Math1*—a Notch signaling pathway transcription factor required for secretory cell fate specification^[26]—resulted in ablation of all intestinal secretory cells, including PCs, leaving the crypt-base populated exclusively by *Lgr5*⁺ CBCs^[24, 25]. In this context, stem cell function and gene expression are maintained,

suggesting PCs are not required to sustain Lgr5⁺ CBCs *in vivo*^[24, 25]. Furthermore, interpretation of PC niche function in the context of Notch ablation is challenging, as potential Notch signaling contributions by PCs are masked. Indeed, Notch signaling between the CBC and PC is likely important; Notch-1 receptor is highly expressed by the Lgr5⁺ CBCs^[27], whereas PCs express the Notch ligands *Dll-1* and *Dll-4*^[15]. These ligands are known to be critical for Notch-mediated homeostasis in the intestinal epithelium, as their genetic removal phenocopies the secretory cell hyperplasia observed upon Notch inhibition^[28]. Despite the evidence of intimate PC-CBC cross-regulation, the EGF expression profile of PCs may also indicate a role for regulating +4 ISCs. The recent identification of a quiescent ISC population marked by the Erb inhibitor Lrig1 points to a potential EGF signaling network within the niche^[7]. Thus, in order to clearly address the controversial PC niche role, future studies must examine the homeostatic contribution of each signaling factor individually. In light of these challenges, Farin and colleagues used a simplified approach to examine the requirement of a single PC-derived ligand, *Wnt3*, within the ISC niche. Tissue specific deletion of *Wnt3* produced no effect *in vivo*, but was required for growth and sustainability *in vitro*^[11]. These disparate requirements of *Wnt3* pointed to a role for functionally redundant Wnt ligands derived from mesenchymal niche components.

Signaling from unidentified mesenchymal cells contributes to ISC homeostasis

The endogenous intestinal niche contains both epithelial and mesenchymal components, which together produce regulatory growth factors, whereas enteroids are derived solely from epithelial cells that are immersed in Matrigel and a growth factor-rich milieu (Figure 1). The strict dependence on PC-derived Wnt *in vitro* strongly suggests the underlying mesenchyme provides compensation for loss of *Wnt3* *in vivo*. Indeed, the growth of *Wnt3*-deficient enteroids was rescued with *Wnt2b* supplementation, a ligand expressed exclusively in the mesenchyme, or by co-culture with adherent mesenchymal cells^[11], directly demonstrating mesenchymal contribution to ISC homeostasis. Currently, a description of the cell census of the mesenchymal niche is incomplete. Multiple mesenchymal cell-types present near the crypt-base are viable niche-cell candidates, most prominently the sub-epithelial myofibroblasts^[3], recently shown to sustain human colonoid cultures^[29]. Wnt expression in specific intestinal mesenchymal cell-types has not been extensively studied and no mesenchymal cell-type is exclusively localized to the crypt-base in the *Wnt2b* expression zone^[14]. The isolation and characterization of mesenchymal niche cells to determine which cells are important in regulating ISCs is outstanding in the field.

Furthermore, the mechanisms that regulate interactions between mesenchymal and crypt-base epithelial cells remain unclear. Signaling between the mesenchyme and epithelial cells is likely bi-directional^[3]. It is possible that crypt-adjacent mesenchymal cells are induced to associate with the niche by signals from the epithelium. Consistently, bone-marrow derived cells home to the base of intestinal crypts after epithelial damage^[30]. Further, a recent study shows that unspecified mesenchymal cells are recruited to sites of intestinal tissue regeneration to attenuate crypt-cell proliferation and restore homeostasis through secretion of the non-canonical ligand, *Wnt5a*^[31]. It is apparent that a better understanding of mesenchymal contributions to the niche is essential and will provide additional insight into mechanisms of homeostasis and disease.

Future prospectives

The work by Farin and colleagues highlights the important compensatory mechanisms hardwired into the niche to safeguard against stem cell dysfunction and disease. It is clear that additional redundancy likely exists *in vivo*, but is not easily uncovered by classical

genetics alone. The complexity of the intact niche complicates dissection of redundant signaling components that function in compensatory upregulation of niche-mediated signaling in response to changes in homeostasis. Although the enteroid culture system is more analogous to the exponential growth observed during tissue regeneration than the *in vivo* homeostatic environment, Farin et al. elegantly demonstrate its utility for elucidating epithelial and mesenchymal signaling contributions to the ISC niche. Understanding the signaling interactions needed for the successful maintenance of the intestine is essential, and the authors fill in another piece of this intricate puzzle.

The identification of novel stem cell markers and the establishment of the *in vitro* intestinal enteroid culture system have revitalized the ISC field. Combined with exciting advances in generating intestinal tissue from human induced pluripotent stem cells^[32] and improved human crypt culturing methods^[29, 33], the potential to expand intestinal tissue *ex vivo* for therapeutic purposes is apparent^[34]. The enteroid system also provides a highly tractable platform for applying molecular techniques^[35] and live-cell imaging to address numerous outstanding questions regarding ISC biology. Future studies will harness this system to uncover additional mesenchymal-epithelial signaling mechanisms in homeostasis and disease, delineate an ISC hierarchy, specify the niche requirements of the +4 ISC population, explore the influence of the microbiome on ISC behavior^[36], identify disease mechanisms and potential therapies, and to develop novel tissue regeneration models. By combining both *in vivo* and *in vitro* approaches, the answers to long-standing questions that have hindered the ISC field are now within reach.

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Figure 1.

Comparison of *in vivo* (left) and *in vitro* (right) signaling support to the intestinal stem cell. Signaling networks between the Paneth cell and stem cell appear to be broadly conserved. However, disruption on one element of a single pathway highlights the important compensation wired into the system where *in vivo* environments can rely upon mesenchymal cells to maintain signaling homeostasis. Wnt, Notch and signaling pathways are key players in the balance between stem cell maintenance and differentiation.