Editorial

Stem Cell INVESTIGATION

Intestinal stem cell resurgence by enterocyte precursors

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The adult intestinal epithelium has a remarkable capacity for renewal that is driven by the activity of stem cells located in the intestinal crypts. Until now two major types of the intestinal stem cells have been identified: (I) actively proliferating intestinal stem cells (aISC), expressing *Lgr5*, that reside at the bottom of the crypts and are fast-cycling and sensitive to injury, e.g., irradiation (1), and (II) quiescent or reserve intestinal stem cells (qISC or rISC), characterized by expression of *Bmi1*, *mTert*, and *Hopx*, that reside at +4 position of the crypt and are slow-cycling and have been shown to facilitate the regeneration of the intestinal epithelium upon injury.

Recent studies have shown that despite divergent characteristics, these two populations of intestinal stem cells display a significant degree of plasticity between them. To further add to the versatile functions of intestinal crypt cells in maintaining epithelial homeostasis, Tetteh *et al.* recently reported that the enterocyte precursors have the ability to dedifferentiate into stem cells upon the ablation of the *Lgr5*$^+$ expressing aISCs (2). They showed that the enterocyte differentiation marker alkaline phosphatase intestinal (*Alpi*) gene is highly expressed not only in the mature enterocytes but in the +6 and +7 positions and coincided with the KI67 proliferative marker in the transit amplifying (TA) zone of the crypts. This finding was critical in identifying *Alpi*$^+$ precursor cells as a unique population within the TA zone that is distinct from *Lgr5*$^+$ aISCs or *Dll1*$^+$ secretory precursors. This further warranted the use of *Alpi* as a proxy to generate enterocyte progenitor lineage tracing mice.

Lineage tracing using an inducible *Alpi*-Cre knock-in and Rosa26-lacZ reporter resulted in the transient appearance of lineage-initiated *Alpi*$^+$ cells originating from the upper part of the crypts. The *Alpi*$^+$ lineage cells were entirely composed of absorptive precursors and enterocytes, and did not include secretory cells, such as goblet, enteroendocrine, and Paneth cells. Furthermore, these lineage cells were soon pushed up by newly dividing and non-initiated precursors and almost completely lost from the epithelium within 6 days following initial induction of the lineage tracing. This demonstrates that during homeostasis *Alpi*$^+$ cells are short-living and do not display multipotency and self-renewal potential.

The authors then sought to investigate whether *Alpi*$^+$ cells are capable of dedifferentiation into stem cells upon injury to aISCs. They used the *Lgr5*$^{DTR\text{-}GFP+/-}$ mouse model which permits ablation of Lgr5$^+$ cells upon treatment with diphtheria toxin. Using this model, they showed that when *Lgr5*$^+$ populations were eradicated from the crypt bottom, *Alpi*$^+$ cells dedifferentiated to stem cells and produced long-lived clones that give rise to both absorptive and secretory cells. Notably, only the *Alpi*$^+$ cells within crypts were capable of dedifferentiating, since depletion of *Lgr5*$^+$ cells three days after *Alpi*$^+$ lineage initiation did not result in long-lasting clones of *Alpi*$^+$ cells.

Previously it has been shown by Tian *et al.* that reserve stem cells expressing *Bmi1* marker can acquire the function of active ISCs, repopulate the intestinal epithelium and, give rise to *Lgr5*$^+$ stem cells (3). The claim that *Bmi1*$^+$ cells are the source of regeneration was further supported using diphtheria toxin- and ionizing radiation-mediated depletion of *Lgr5*$^+$ stem cells (1,3). The regenerative potentials of reserve ISCs following aISC depletion have been further validated using other markers *Hopx* and *mTert* (4,5). Additionally, *Dll1*$^+$ secretory precursors (6) and *Krt19*$^+$ cells (7) have been previously shown to rescue intestinal epithelium upon *Lgr5*$^+$ cell deletion

using ionizing radiation. Buczacki *et al.* suggested that quiescent cells at +4 position are committed secretory precursors, which may provide explanations to various cell populations assuming the role of active ISCs (8). However, crypt cells undergo dynamic proliferation and changes in positions, and the specificity of the reserve ISC markers remain in flux. A recent study using *Bmi1*- and *Hopx-CreER* mouse models has shown that *Bmi1* and *Hopx* expressions largely overlap with one another, and this cell population is molecularly and functionally distinct from secretory precursors at +4 position (9). Additionally, a zinc-finger transcription factor KLF4, which is also a radioprotective factor, is expressed in a subpopulation of *Bmi1*$^+$ cells, rendering antiapoptotic and regenerative functions to these cells upon intestinal injury by ionizing radiation (10,11). These results further support the regenerative role of reserve ISCs in intestinal epithelium. Collectively, these results depict an extraordinary phenomenon, that multiple types of crypt cells are potential reservoirs for stem cells when active ISCs are depleted with varying degree of responses depending on the nature of injuries. The lineage tracing studies of various markers highlight the phenomenal plasticity and interplay of crypt cells in maintaining epithelial homeostasis upon injuries that deplete *Lgr5*$^+$ stem cells' population.

To investigate whether the transcriptome of *Alpi*$^+$ cells undergoes modifications upon *Lgr5*$^+$ cell depletion and gains characteristics of stem cells, Tetteh *et al.* performed single-cell RNA sequencing of short-term labeled *Alpi*$^+$ cells during homeostasis and following *Lgr5*$^+$ cell depletion. They then employed a rare cell type identification (RaceID) algorithm to analyze the transcriptome data. RaceID is an algorithm developed by Grün *et al.* to identify rare cells within heterogeneous populations of cells with abundance cell types (12). Using RaceID analysis, Tetteh *et al.* identified five distinct clusters of cells based on enrichment of specific transcripts. In homeostatic condition without *Lgr5*$^+$ cell depletion, *Alpi*$^+$ lineage cells were exclusive to cluster 1 with enriched enterocyte-specific genes such as *Apoa1* and *Fabp2*. *Alpi*$^+$ lineage cells of *Lgr5*$^+$ depleted crypts displayed more diverse populations that the authors identified as regenerating enterocytes (*Alpi*, *Fth1*, *Fabp2)*, proliferative cells (*Ascl2*, *Smoc2*, *Cdca7)*, Paneth-like cells (*Lyz1*, *Defa1*7, *Mmp7)*, and unknown cells expressing rare secretory cell marker genes.

ASCL2 is a WNT-dependent transcription factor restrictively expressed in intestinal stem cells. Identification by authors of a cell cluster that expresses *Ascl2* and ribosomal proteins suggest that *Alpi*$^+$ lineages are dedifferentiating to stem cells. Paneth cells are interspersed between *Lgr5*$^+$ cells at the crypt bottom, where they contribute to creating stem cell niche (13). The emergence of Paneth-like cell populations from *Alpi*$^+$ lineage in *Lgr5*$^+$ depleted cells may be attributed to the intestinal epithelium recognizing the loss of active ISCs, thus creating a microenvironment that would stimulate the resurgence of stem cells from other cell populations. One interesting follow-up experiment would be to localize the positions of Paneth-like cells in regenerating crypts and determine whether directly adjoining cells are more likely to express stem cell markers, such as *Ascl2*.

Although *Alpi*$^+$ cells have a rapid proliferation rate during homeostasis and can acquire stem cell functions in absence of *Lgr5*$^+$ stem cells, loss of *Apc* or gain of *Kras*^{G12D} mutations in *Alpi*$^+$ cells did not result in tumor formation *in vivo*. The authors discussed that the transient localization of *Alpi*$^+$ precursors in crypt prevent these cells from becoming tumor cells, since *in vitro* organoids cultured without ISC-required growth factors using these cells exhibited tumor-like hyperproliferation. It is important to take into account that intestinal crypts for organoid cultures undergo tissue damage during the collection process, and this may affect cellular processes and behaviors. Despite this possibility, the protective aspect of transient localization and dynamic mobilization of highly proliferative absorptive precursors is evolutionarily beneficial for the well-being of our gut system.

Tetteh *et al*. demonstrated the existence of a new population of cells (*Alpi*$^+$) with the capability to gain "stemness" and convert into *Lgr5*$^+$ aISC upon ablation of the original population of aISC. This study is important as it contributes to the current understanding that there is an astonishing degree of plasticity within the cells of the intestinal crypts that allows regeneration upon injury to the *LGR5*$^+$ stem cell population. Cancer therapies, such as radiation and chemotherapy, induce DNA damages in highly proliferative cells, including cancer cells and normal stem cells. In addition to these therapeutic side effects, other intestinal epithelial damages without discriminatory cell population targets, such infections and autoimmune diseases, require global regenerative responses. While the absorptive precursors are also highly proliferative, thus may be considered fragile in nature, they still make up the majority of the crypt cell population, providing an abundant pool of stem cell replacements if and when necessary. This is significantly advantageous to maintaining homeostasis

of intestinal epithelium. As a follow up, determining the difference in caliber of conversion by aISC candidates of the crypt in the regenerative process would identify therapeutically targetable key contributors.

Beyond the recognition of specific population of crypt cells with capability to self-renewal under various conditions, more focus must be placed on determining the mechanism(s) by which that cellular transformation of crypt cells into active ISCs is established. Reasonably, only *Alpi*$^{+}$ enterocytes cells within the crypt compartment can replace aISCs. Since the location of the intestinal epithelial cells may serve as an indicator of cell maturity, the differential profiling of cellular and molecular factors and processes involved in crypt cell maturation to differentiated cells is an important next step. Ultimately, this will lead to identifying potential therapeutic targets that modulates intestinal epithelial regeneration upon injuries.

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