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EDITORIAL

Decrypting the Crypt: Novel Monoclonal Antibodies to Identify Intestinal Stem Cell Populations

Despite extensive efforts to fully characterize and identify the stem cell pools at the base of the small intestinal crypts, there remains controversy over the precise identity of these cells. The active crypt base columnar cell, labeled by leucine-rich G-protein–coupled receptor 5 (Lgr5), is the workhorse of epithelial renewal, but other cell populations are able to contribute to this renewal, particularly after intestinal damage. The accurate labeling of these additional stem cell populations is plagued by the small numbers of these cells and the plasticity of the cell itself. Even labeling of the active stem cell population by Lgr5-*enhanced green fluorescent protein* (EGFP) has been imperfect, with mosaic silencing in the commonly used Lgr5$^{\text{EGFP-internal ribosome entry site (IRES)-Cre-estrogen receptor binding domain fusion (IRES-Cre-ER)}}$ transgenic mouse, and concurrent expression of markers of other stem cell populations observed in these active intestinal stem cells (ISCs). In addition, the use of transgenes to label intestinal stem cells cannot be applied to human beings and therefore direct application of findings in these models can be difficult.

In the recent publication by Smith et al[1] the researchers confronted this problem by raising antibodies to various intestinal epithelial populations, then screening these for immunoreactivity to identified cells using a variety of methods. The investigators were successfully able to prove, via single-cell growth analyses, immunofluorescence, and gene expression, that the monoclonal antibody (mAb) F5C12 marks a population with essentially identical properties to Lgr5$^{\text{GFP}}$ sorted cells. Notably, the F5C12 mAb does not mark slow-cycling crypt cells or villous epithelium. A second mAb, E5D10, was highly expressed on villous epithelium, with low expression present in the crypt. Therefore, E5D10$^{\text{lo}}$/F5C12^{-} sorted cells contain all other crypt cells that are not actively cycling ISCs.

Although Smith et al[1] showed that this population included Bmi1$^{\text{GFP}}$ cells with the ability to form spheroids in culture, they did comment that it also included Paneth cells, as indicated by higher lysozyme messenger RNA expression. It would have been interesting to further discriminate the E5D10$^{\text{lo}}$/F5C12^{-} population by ulex europaeus agglutinin (UEA)$^{+}$ or side-scattered light (SSC)$^{\text{hi}}$ to remove the influence of concurrently isolated Paneth cells. However, because the *Bmi1*-expressing cell population has been reported to be insensitive to *Wnt* signaling, removal of the epithelial source of *Wnt* is unlikely to have altered the conclusions of the study. This also was supported by the differential response of the subsequent ex vivo cultures to the lack of R-spondin1, in which enteroids produced by actively cycling cells (F5C12^{+}) were unable to survive the loss of *Wnt* enhancement, but the E5D10$^{\text{lo}}$/F5C12^{-} spheroids continued to proliferate.

Bmi1$^{\text{GFP}}$ cells have been identified previously as committed enteroendocrine precursors that retain the ability to dedifferentiate into an active stem cell. This functionality likely relies on a facile alteration of their chromatin signature back to an active ISC profile. Disappointingly, Bmi1$^{\text{Cre-estrogen receptor binding domain fusion (IRES-Cre-ER); tandem dimeric tomato}}$ marks more cells than just those marked by Bmi1$^{\text{GFP}}$, which makes lineage tracing from these cells difficult to reconcile with the signatures of the Bmi1$^{\text{GFP}}$-expressing cells. This study used Bmi1$^{\text{CreERT2;tdTom}}$ only in the ex vivo cultures of E5D10$^{\text{lo}}$/F5C12^{-} flow sorted cells, which the investigators had identified previously as broadly expressing Bmi1$^{\text{GFP}}$. Therefore, the Bmi1$^{\text{CreERT2;tdTom}}$ cells, when isolated by E5D10$^{\text{lo}}$/F5C12^{-}, likely represent the overlap in populations identified by the Bmi1$^{\text{GFP}}$ and Bmi1$^{\text{CreERT2;tdTom}}$ transgenes. As such, this E5D10$^{\text{lo}}$/F5C12^{-} population represents cells possessing the ability to restore stem cell function ex vivo.

By using these novel mAbs to isolate different populations for ex vivo culture, the investigators showed a temporal plasticity in ISC gene expression. That is, isolated actively cycling ISCs (F5C12^{+}) did not express Lgr5, as indicated by messenger RNA and by loss of Lgr5$^{\text{GFP}}$ expression, from 2 to 5 days after placement into culture. In addition, lineage tracing of Lgr5$^{\text{GFP-IRES-CreER}}$ cells, when induced with tamoxifen at day 2, showed minimal lineage tracing events. Instead, these ex vivo cultures had increased levels of *Bmi1* transcripts on day 2. The investigators speculate that, when grown from isolated single cells, actively cycling stem cells transiently express a transcript profile consistent with slow-cycling stem cells. This coincides with the phenotypic appearance of these F5C12^{+} cultures: more spheroid than enteroid. Although this finding provides an exciting insight into the plasticity of these stem cell populations, it remains to be seen whether this holds true in vivo. Finally, if the F5C12 mAb continued to mark these cells as they transition through a slow-cycling-like phase, then the true utility of this mAb lies in the ability to follow active intestinal stem cells through damage and repair phases.

This article represents an exciting and novel approach to isolation and identification of separate pools of intestinal stem cells. Furthermore, it describes potentially valuable tools for use in studies using transgenic and nontransgenic mice, large animal models, and human beings.

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