Commentary

Stem Cell INVESTIGATION

Chromatin silencing maintains the identity of intestinal stem cells

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The epithelial sheet of the small intestine harbors villi structures in the lumen and invaginations to form the crypts of Lieberkühn (1). Intestinal stem cells (ISCs) at the bottom of the crypts give rise to Paneth cells and transit-amplifying cells that are capable of differentiating into different types of cells that are components of the small intestine (1). Hierarchies of stem cell models suggest that there are 4 to 6 stem cells per crypt, which decide the ultimate pattern of the entire crypt (2). Two models were originally established for ISCs: the +4 label retaining cell (LRC) model and the crypt base columnar cell (CBC) or Lgr5$^+$ cell model (2,3). Wnt signaling is known to be essential for Lgr5$^+$ ISCs, and the bottom of the crypt is thought to be a stem cell niche in which Wnt signaling crosstalks with other signaling pathways in regulating self-renewal of ISCs as well as their differentiation, proliferation and migration (4).

At the +4 position of the crypt, an ISC marker, B lymphoma Mo-MLV insertion region 1 (Bmi1), is predominantly expressed (5). Bmi1 belongs to the Polycomb group (PcG) gene family, which functions in gene silencing through chromatin modifications. Through lineage analysis of repopulation kinetics of the *Bmi1*$^+$ lineage, Sangiorgi *et al.* demonstrated that Bmi1-expressing stem cells are distributed along the length of the small intestine and suggested that mice use more than one adult stem cell subpopulation to maintain organ homeostasis (5). However, the precise role of polycomb group proteins in maintaining ISCs was not defined.

Recent study by Chiacchiera *et al.* demonstrated that the Polycomb repressive complex PRC1 plays a master role in maintaining homeostasis of the intestinal epithelium (6) (*Figure 1*). By coupling a constitutive knockout (KO) allele for *Ring1a* and a 4-hydroxytamoxifen (OHT)-inducible Cre-dependent KO allele for *Ring1b* in the mouse model, Chiacchiera *et al.* acutely inactivated PRC1 and observed a rapid loss of body weight coupled with a thinner intestine with impaired function in the mice. Loss of H2A monoubiquitination was due to loss of PRC1 activity, which played a direct role in controlling intestinal homeostasis in the adult mice (6).

Using the ISC-specific *Lgr5*$^{GFP\text{-}CreERT2}$ mouse model created by H. Clevers laboratory (7), Chiacchiera *et al.* investigated the role of PRC1 in H2A monoubiquitination and homeostasis in the intestine. They found that ISC-specific PRC1 ablation reduced ISC number and affected normal crypt architecture. *Ring1a/b* double KO mice showed degenerating crypts starting from 7 days post tamoxifen induction, and the number of these abnormal crypts increased after 15 days. All the degenerating crypts stained negative for ubiquitinated H2A. Fluorescence-activated cell sorting (FACS) analysis of these crypts showed a remarkable reduction of GFP+ ISCs, further demonstrating that loss of PRC1 leads to a robust reduction of the ISC pool in mouse crypts. Analysis in the cell spheroid culture also showed the essential role of PRC1 in preserving the homeostasis of the adult intestinal epithelium by maintaining ISC self-renewal independently from their niche (6).

Chiacchiera *et al.* further investigated whether PRC1 has a direct role in the maintenance of intestinal identity in the stem cells. By comparing both up- and down-regulated genes with the transcriptional profiles from different tissues, they found that PRC1 inactivation leads to loss of general intestinal lineage identity rather than to differentiation of ISCs. Using ChIP-seq, Chiacchiera *et al.* identified Ring1b- and H2Aubq-enriched genomic loci in both crypts and ISCs. RNA-seq analysis in the *Ring1a/b* double KO villi confirmed that PRC1 is required to maintain transcriptional repression in ISCs (6).

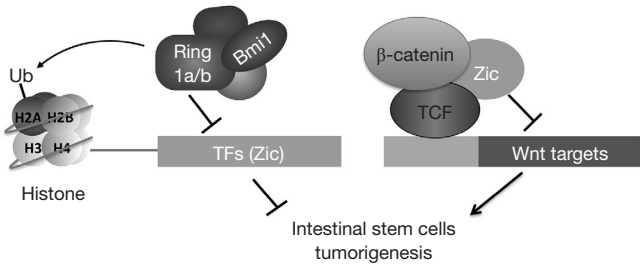

Figure 1 Polycomb complex PRC1 controls the identity of intestinal stem cells (ISCs). PRC1-mediated chromatin silencing on Zic and other transfection factors (TFs) maintains the activity of Wnt signaling required for ISC self-renewal and colon tumorigenesis.

Analysis of gene ontology databases identified activation of DNA-binding transcription factors (TF) in *Ring1a/b* double KO ISCs, with the main function of PRC1 in suppressing the transcription of these genes in ISCs. Among the identified genes, Chiacchiera *et al.* demonstrated that simultaneous inhibition of Zic1 and Zic2 expression in HCT116 enhanced β-catenin/TCF transcriptional activity, while independent expression of either Zic1 or Zic2 induced intestinal organoid regression similarly to loss of PRC1 activity. Chiacchiera *et al.* further demonstrated that Zic1 or Zic2 expression inhibited β-catenin/TCF transcriptional activity via direct interaction with TCF7L2, thus affecting tissue homeostasis in the organoids. This model was further validated in mouse studies by combining β-catenin activation with loss of PRC1 activity in ISCs (6).

A previous study by Yu *et al.* suggested that Wnt signaling also regulates Bmi1 expression in normal intestine and colon cancer (8). Wnt signaling enhances c-Myc expression and c-Myc directly binds to Bmi1 promoter and activates its transcription. A Wnt repressor protein, KLF4, inhibited the expression of PRC proteins including Bmi1 and Ring1B and reduced histone monoubiquitination (8). Bmi1 is overexpressed in human colon cancers, and knocking down Bmi1 by shRNAs inhibited colon cancer xenografts in nude mice (8). The study by Chiacchiera *et al.* further explained the mechanism of how Bmi1 and other PRC proteins regulate adult stem cells in the intestine (*Figure 1*).

The above studies suggest that the PRC1 complex is a valuable drug target for colon cancer treatment. In fact, Kreso *et al.* reported that treatment of primary colorectal cancer xenografts with a small-molecular Bmi1 inhibitor led to reduced levels of histone monoubiquitination and loss of cancer initiating cells (9). This Bmi1 inhibitor represses the expression of Bmi1; it will be interesting to develop other small-molecular inhibitors targeting PRC1 activity in terms of histone ubiquitination.

Study by Chiacchiera *et al.* demonstrated that chromatin silencing could repress the expression of transcription factors that inhibit Wnt signaling and thus sustain the activity of Wnt signaling in the ISCs. The intestine stem cell is a fantastic research area; there are still many avenues to explore. For example, what are the roles of other transcription factors repressed by PRC1 and what are the different roles of PRC1 in $Lgr5^{+}$ ISCs and +4 ISCs? Zic has been reported as both positive and negative regulator of Wnt (10); further investigation of the mechanism and function of Zic in the intestine may lead to other unexpected findings.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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