

# Polycomb complex PRC1 as gatekeeper of intestinal stem cell identity

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**Abstract:** Intestinal stem cells (ISCs) are adult multipotent cells essential for the maintenance of intestinal epithelial homeostasis. Wnt signaling activity ensures that the pool of ISCs at the basis of the intestinal crypts is preserved. Dysregulation of the Wnt pathway is often observed in cancer and supports malignant progression. Chiacchiera and colleagues recently demonstrated the implication of the polycomb complex PRC1 in the regulation of the Wnt pathway in adult ISCs. The authors show that PRC1 maintains intestinal homeostasis by repressing the expression of ZICs, a family of transcription factors inactivating the  $\beta$ -catenin/TCF complex. Importantly, interfering with PRC1 activity completely inhibits the formation of Wnt-dependent tumors. These findings reveal a new layer of epigenetic regulation of the Wnt pathway and open novel opportunities for cancer stem cell targeted therapy.

**Keywords:** Polycomb complex PRC1; intestinal stem cells (ISCs); Wnt pathway

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Stem cells are key players in development, maintenance and regeneration of adult tissues. In the small intestine, stem cells reside at the bottom of the crypt, interspersed with Paneth cells, where they divide to give rise to progenitor or transiently amplifying (TA) cells (1). After few division rounds, TA cells differentiate and concomitantly ascend along the crypt-villus axis, to eventually complete the renewal of the intestinal epithelium in 4–5 days. The maintenance of tissue homeostasis relies on a variety of factors and signaling pathways operating in the intestinal crypt and necessary for the generation of the stem cell niche (2). The Wnt signaling is of particular interest as it is a well-conserved pathway controlling self-renewal and lineage commitment in metazoans. In vertebrates, 19 different Wnt proteins ligands are involved in the spatio-temporal activation of multiple signaling pathways (3). In particular, activation of the canonical Wnt pathway via Frizzled (Fzd) and its co-receptors LRP5/6 tethers to the plasma membrane the multiprotein destruction complex, consisting of adenomatous polyposis coli (APC), Axin,

glycogen synthase kinase 3 (GSK-3), casein kinase 1 (CK1), protein phosphatase 2A (PP2A) and E3-ubiquitin ligase  $\beta$ -TrCP, which results in the cytoplasmic accumulation and nuclear translocation of  $\beta$ -catenin. In the nucleus,  $\beta$ -catenin regulates the transcription of a subset of genes in cooperation with members of the TCF/LEF family of transcription factors. While the integrity of this pathway is essential for the ISC self-renewal and for the homeostasis of intestinal epithelium, its deregulation is often observed in diseases such as cancer. Loss-of-function of Wnt negative regulators has been extensively investigated. For instance, mutations in the *APC* gene are found in colorectal or gastric cancer, truncating or missense mutations in the *Axin* genes are associated with colorectal and hepatocellular cancer, and missplicing of *GSK3 $\beta$*  transcript is prevalent in patients with leukemia (4–6). However, gain-of-function mutations, for instance in the  *$\beta$ -catenin* and *TCF7L2* genes have also been observed in cancer, specifically in hepatocellular carcinoma and colorectal cancer (7,8).

The epigenetic control of the Wnt signaling pathway

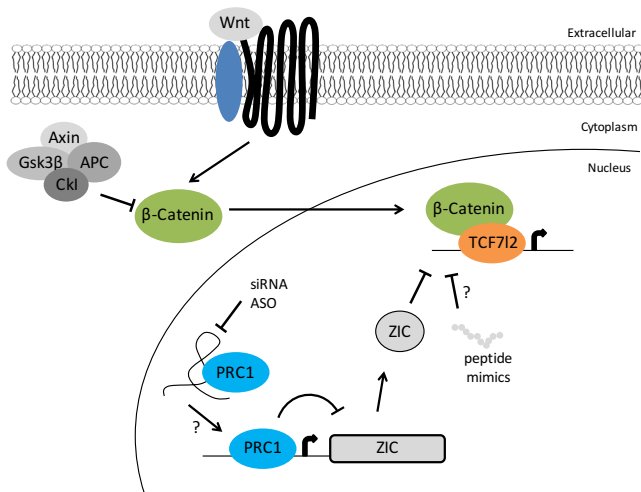
lately emerged as an important additional regulatory layer. Indeed, several reports already highlighted the epigenetic inactivation of Wnt pathway negative regulators, including DACH1, WIF1, SFRP and DKKs, in various human malignancies (9-12). Recently, Chiacchiera and colleagues demonstrated that the Polycomb repressive complex 1 (PRC1) is required for the integrity of the intestinal epithelium by investigating its function in adult intestinal stem cells (13). The polycomb group proteins are important epigenetic regulators involved in development and pluripotency (14). PRC1 complexes display a great plasticity in eukaryotes with at least six biochemically distinct groups (15). All these complexes operate in collaboration with the E3 ubiquitin ligases Ring1a or Ring1b, which catalyse the mono-ubiquitination of H2A (H2Aubq) on lysine 119 (H2AK119). To characterize the function of PRC1 in adult tissue, the authors employed a mouse model that couples a constitutive knockout (KO) of *Ring1a* with a conditional KO of *Ring1b*. While *Ring1a* KO is largely compensated by *Ring1b* expression, inactivation of both genes severely hindered intestinal homeostasis as well as H2A ubiquitination. Moreover, loss of PRC1 function in Lgr5-ISCs generates a gradual deterioration of the crypt architecture (13). This finding echoes numerous earlier reports supporting the notion that polycomb group proteins maintain stem cell renewal by repressing genes that control differentiation (16-21). Next, the authors investigated whether *Ring1a/Ring1b* double KO cells could properly differentiate, by monitoring the activity of alkaline phosphatase in the villi. Tissue staining revealed that double KO cells were unable to complete differentiation of specialized intestinal cells as compared to wild-type ISCs. Interestingly, this was associated with the downregulation of key transcription factors involved in this process (e.g., *Ascl2*, *gata4/6* and *cdx1/2*). However, in accordance with the repressive function of PRC1, most of the differentially regulated genes in *Ring1a/Ring1b* double KO cells were upregulated. Further analysis of differentially expressed genes revealed that disruption of PRC1 in ISCs prevents proper intestinal lineage commitment rather than promoting a premature cellular differentiation (13). The notion that PRC1 serves as a gatekeeper to block inadequate lineage commitment was already introduced in other systems. For instance, knockdown of 2 different components of PRC1 complex (BMI1 and Ring1b) in myoblast impedes the formation of fully differentiated myotubes (22). Similarly, loss of PRC1 function through inactivation of KDM2B and CBX4 blocks the lineage

commitment of hematopoietic stem cells and epidermal basal cells, respectively (23,24).

The finding that PRC1 has the ability to establish different repressive transcriptional programs raises questions about the mechanisms used by cells to specifically recruit the complexes to the chromatin. Genome-wide profiling studies demonstrated that PRC1 and PRC2 polycomb complexes share a subset of target sites (17,20). Characterization of PRC1 and PRC2 recruitment to these targets revealed that inactivation of PRC2 (with consequent loss of histone 3 lysine 27 trimethylation, H3K27me3), determines loss of PRC1 binding to target sites (25). This suggests that PRC1 is sequentially recruited to the DNA after the binding of PRC2 and the deposition of H3K27 methylation. However, this classical model does not explain the PRC2-independent tethering of PRC1 to other genomic loci. Recent reports demonstrated that PRC1 could be recruited to CpG islands via binding to the lysine demethylase 2B or KDM2B (26,27). In this view, contrary to the classical model, PRC1 recruitment by KDM2B allows the sequential binding of PRC2 and the silencing of gene expression. Alternatively, PRC1 can be recruited to the DNA through interaction with non-coding RNAs (ncRNAs) (28-31). PRC1 was found to interact with ncRNAs via its subunit SCML2A (31). Interestingly, interfering with this interaction by mutating the RNA binding region of SCML2 strongly reduced PRC1 recruitment to the chromatin, thus supporting the role of ncRNAs as potential chromatin anchoring points for protein complexes.

To further clarify how PRC1 supports ISC self-renewal, Chiacchiera and colleagues performed a gene ontology analysis on upregulated genes identified in *Ring1a/Ring1b* double KO cells. The authors found that the only significantly enriched functional ontology class was DNA-binding transcription factors. Interestingly, among these, the *Zic* family of zinc transcription factors was previously reported to interfere with the Wnt pathway (32). Using a combination of ectopic expression/inactivation of ZICs, and immunoprecipitation experiments in colorectal cancer cell lines or intestinal-crypt derived organoids, the authors showed that ZIC1 and ZIC2 can inhibit the Wnt signaling by physically interacting with the  $\beta$ -catenin/TCF complex (13). In accordance with this hypothesis, the genomic occupancy of TCF7L2 in *Ring1a/Ring1b* double KO cells is reduced. Thus, PRC1 activity is required to repress the expression of ZIC TFs, and therefore sustain Wnt signaling in ISCs.

In their report, Chiacchiera and coworkers proved the existence of a PRC1-dependent epigenetic control



**Figure 1** Schematic representation of new potential approaches to interfere with the Wnt pathway. In intestinal stem cells Wnt ligands determine the accumulation of  $\beta$ -catenin in the cytoplasm and in the nucleus, by counteracting the destruction complex (i.e., APC, Axin, Gsk3 $\beta$  and Ckl). In the nucleus, PRC1 sustain  $\beta$ -catenin activity by repressing the expression of ZIC transcription factors. Inactivation of putative lncRNAs guiding or anchoring PRC1 at ZIC promoter, as well as the use of peptides mimicking ZIC protein function, might represent promising strategies against Wnt-dependent tumors.

of the Wnt signaling pathway in ISCs. This newly discovered regulatory layer operates via the transcriptional repression of ZIC TFs, and prevents the formation of the transcriptionally inactive ZIC/ $\beta$ -catenin/TCF complex. Thus, PRC1 activity maintains the pool of ISCs and is essential for normal intestinal tissue homeostasis. Interestingly, this epigenetic control could potentially contribute to the dysregulation of Wnt/ $\beta$ -catenin signaling pathway often observed in human diseases such as cancer (33-35). Indeed, loss of PRC1 activity in tumor cells derived from ISCs expressing a mutant form of  $\beta$ -catenin (*CTNNTB1<sup>ex3/ex3</sup>*) fully prevents tumor formation (15). These findings highlight the potential of blocking PRC1 activity as a new therapeutic approach to target cancer stem cells, which rely on Wnt signaling for growth and survival. While efforts to block PRC1 activity using a small inhibitory molecule have already been reported (36), another possibility might be the use of peptides able to mimic ZIC function and therefore displace  $\beta$ -catenin/TCF complex from the chromatin. However, deleterious effects on healthy stem cells and tissues homeostasis are certainly

a potential drawback to consider before therapeutics aimed at blocking PRC1 or the Wnt signaling can be developed. Alternatively, the emerging involvement of lncRNAs as guides or chromatin docking intermediates for protein complexes might offer unique opportunities to interfere with specific PRC1 complexes and, therefore, minimize unwanted effects (Figure 1) (37,38).

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## Footnote

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

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## References

1. Barker N. Adult intestinal stem cells: critical drivers of epithelial homeostasis and regeneration. *Nat Rev Mol Cell Biol* 2014;15:19-33.
2. Losick VP, Morris LX, Fox DT, et al. *Drosophila* stem cell niches: a decade of discovery suggests a unified view of stem cell regulation. *Dev Cell* 2011;21:159-71.
3. van Amerongen R, Nusse R. Towards an integrated view of Wnt signaling in development. *Development* 2009;136:3205-14.
4. Clements WM, Lowy AM, Groden J. Adenomatous polyposis coli/beta-catenin interaction and downstream targets: altered gene expression in gastrointestinal tumors. *Clin Colorectal Cancer* 2003;3:113-20.
5. Salahshor S, Woodgett JR. The links between axin and carcinogenesis. *J Clin Pathol* 2005;58:225-36.
6. Abrahamsson AE, Geron I, Gotlib J, et al. Glycogen synthase kinase 3beta missplicing contributes to leukemia stem cell generation. *Proc Natl Acad Sci U S A* 2009;106:3925-9.
7. Polakis P. The many ways of Wnt in cancer. *Curr Opin*

- Genet Dev 2007;17:45-51.
8. Cuilliere-Dartigues P, El-Bchiri J, Krimi A, et al. TCF-4 isoforms absent in TCF-4 mutated MSI-H colorectal cancer cells colocalize with nuclear CtBP and repress TCF-4-mediated transcription. *Oncogene* 2006;25:4441-8.
  9. Yan W, Wu K, Herman JG, et al. Epigenetic regulation of DACH1, a novel Wnt signaling component in colorectal cancer. *Epigenetics* 2013;8:1373-83.
  10. Batra S, Shi Y, Kuchenbecker KM, et al. Wnt inhibitory factor-1, a Wnt antagonist, is silenced by promoter hypermethylation in malignant pleural mesothelioma. *Biochem Biophys Res Commun* 2006;342:1228-32.
  11. Suzuki H, Watkins DN, Jair KW, et al. Epigenetic inactivation of SFRP genes allows constitutive WNT signaling in colorectal cancer. *Nat Genet* 2004;36:417-22.
  12. Maehata T, Taniguchi H, Yamamoto H, et al. Transcriptional silencing of Dickkopf gene family by CpG island hypermethylation in human gastrointestinal cancer. *World J Gastroenterol* 2008;14:2702-14.
  13. Chiacchiera F, Rossi A, Jammula S, et al. Polycomb complex PRC1 preserves intestinal stem cell identity by sustaining Wnt/ $\beta$ -catenin transcriptional activity. *Cell Stem Cell* 2016;18:91-103.
  14. Gil J, O'Loughlin A. PRC1 complex diversity: where is it taking us? *Trends Cell Biol* 2014;24:632-41.
  15. Gao Z, Zhang J, Bonasio R, et al. PCGF homologs, CBX proteins, and RYBP define functionally distinct PRC1 family complexes. *Mol Cell* 2012;45:344-56.
  16. Tolhuis B, de Wit E, Muijters I, et al. Genome-wide profiling of PRC1 and PRC2 Polycomb chromatin binding in *Drosophila melanogaster*. *Nat Genet* 2006;38:694-9.
  17. Boyer LA, Plath K, Zeitlinger J, et al. Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature* 2006;441:349-53.
  18. Landeira D, Sauer S, Poot R, et al. Jarid2 is a PRC2 component in embryonic stem cells required for multi-lineage differentiation and recruitment of PRC1 and RNA Polymerase II to developmental regulators. *Nat Cell Biol* 2010;12:618-24.
  19. He J, Shen L, Wan M, et al. Kdm2b maintains murine embryonic stem cell status by recruiting PRC1 complex to CpG islands of developmental genes. *Nat Cell Biol* 2013;15:373-84.
  20. Ku M, Koche RP, Rheinbay E, et al. Genomewide analysis of PRC1 and PRC2 occupancy identifies two classes of bivalent domains. *PLoS Genet* 2008;4:e1000242.
  21. Fouse SD, Shen Y, Pellegrini M, et al. Promoter CpG methylation contributes to ES cell gene regulation in parallel with Oct4/Nanog, PcG complex, and histone H3 K4/K27 trimethylation. *Cell Stem Cell* 2008;2:160-9.
  22. Asp P, Blum R, Vethantham V, et al. Genome-wide remodeling of the epigenetic landscape during myogenic differentiation. *Proc Natl Acad Sci U S A* 2011;108:E149-58.
  23. Andricovich J, Kai Y, Peng W, et al. Histone demethylase KDM2B regulates lineage commitment in normal and malignant hematopoiesis. *J Clin Invest* 2016;126:905-20.
  24. Mardaryev AN, Liu B, Rapisarda V, et al. Cbx4 maintains the epithelial lineage identity and cell proliferation in the developing stratified epithelium. *J Cell Biol* 2016;212:77-89.
  25. Cao R, Wang L, Wang H, et al. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science* 2002;298:1039-43.
  26. Blackledge NP, Farcas AM, Kondo T, et al. Variant PRC1 complex-dependent H2A ubiquitylation drives PRC2 recruitment and polycomb domain formation. *Cell* 2014;157:1445-59.
  27. Farcas AM, Blackledge NP, Sudbery I, et al. KDM2B links the Polycomb Repressive Complex 1 (PRC1) to recognition of CpG islands. *Elife* 2012;1:e00205.
  28. Yap KL, Li S, Muñoz-Cabello AM, et al. Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a. *Mol Cell* 2010;38:662-74.
  29. Hu X, Feng Y, Zhang D, et al. A functional genomic approach identifies FAL1 as an oncogenic long noncoding RNA that associates with BMI1 and represses p21 expression in cancer. *Cancer Cell* 2014;26:344-57.
  30. Puvvula PK, Desetty RD, Pineau P, et al. Long noncoding RNA PANDA and scaffold-attachment-factor SAFA control senescence entry and exit. *Nat Commun* 2014;5:5323.
  31. Bonasio R, Lecona E, Narendra V, et al. Interactions with RNA direct the Polycomb group protein SCML2 to chromatin where it represses target genes. *Elife* 2014;3:e02637.
  32. Fujimi TJ, Hatayama M, Aruga J. *Xenopus* Zic3 controls notochord and organizer development through suppression of the Wnt/ $\beta$ -catenin signaling pathway. *Dev Biol* 2012;361:220-31.
  33. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol*

- 2004;20:781-810.
34. Reya T, Clevers H. Wnt signalling in stem cells and cancer. *Nature* 2005;434:843-50.
  35. Klaus A, Birchmeier W. Wnt signalling and its impact on development and cancer. *Nat Rev Cancer* 2008;8:387-98.
  36. Ismail IH, McDonald D, Strickfaden H, et al. A small molecule inhibitor of polycomb repressive complex 1 inhibits ubiquitin signaling at DNA double-strand breaks. *J Biol Chem*. 2013;288:26944-54.
  37. Guttman M, Rinn JL. Modular regulatory principles of large non-coding RNAs. *Nature* 2012;482:339-46.
  38. Morris KV, Mattick JS. The rise of regulatory RNA. *Nat Rev Genet* 2014;15:423-37.

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