OUTLOOK

A protein synthesis brake for hematopoietic stem cell maintenance

Kaosheng Lv[1] and Wei Tong[2]

[1]Department of Biochemistry, School of Medicine, Southern University of Science and Technology, Shenzhen, Guangdong 518000, China; [2]Children's Hospital of Philadelphia; Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

Bmi1 is essential for normal and leukemic hematopoiesis, but its target genes in hematopoietic stem cells (HSCs) are incompletely understood. In this issue of *Genes & Development*, Burgess et al. (pp. 887–900) demonstrate a novel role of Bmi1 in regulating ribosome biogenesis and protein synthesis. *Bmi1*-deficient HSCs exhibited reduced transplantability, with the up-regulation of ARX and genes involved in ribosome biogenesis. However, depletion of *ARX* or its known targets, $p16^{Ink4a}/p19^{Arf}$, only partially rescues *Bmi1* loss-induced hematopoietic defects. They further demonstrate an increased protein synthesis rate and resultant proteostatic stress in $Bmi1^{-/-}$ HSCs, indicating a novel mechanism by which Bmi1 controls HSC maintenance.

Proteostasis is now recognized to play a crucial role in hematopoietic stem cell maintenance and function (Tahmasebi et al. 2019; Chua and Signer 2020). Hematopoietic stem cells (HSCs) exhibit a low protein synthesis rate to maintain their stemness (Signer et al. 2014). Multiple intrinsic mechanisms orchestrate this elaborate process within stem cells, including ribosome biogenesis and assembly, RNA modifications, the ubiquitin–proteasome system, and other post-translational modifications. Among these, ribosome biogenesis and assembly have been underscored by their etiological connection to human diseases, as exemplified by bone marrow failure syndromes (Kang et al. 2021). Moreover, several studies suggest that targeting ribosome biogenesis and protein homeostasis shows promising therapeutic efficacy in improving HSC function (Kruta et al. 2021; Lv et al. 2021).

In addition to the aforementioned mechanisms, transcription factors and epigenetic regulators are found to control HSC function by targeting ribosome-mediated protein translation (Cai et al. 2015; Nachmani et al. 2019). Bmi1 is an essential component of Polycomb repressive complex 1 (PRC1). This complex acts predominantly as a transcription repressor, but also possesses transcription activation function under specific circumstances (Geng and Gao 2020). Bmi1 is crucial for HSC self-renewal and balanced lineage differentiation (Park et al. 2003; Oguro et al. 2010). However, the downstream effectors of Bmi1 in regulating HSC function remain incompletely understood.

In this issue of *Genes & Development*, Burgess et al. (2022) did extensive analyses to define the underlying mechanism (Fig. 1). Due to the early death of *Bmi1* germline knockout mice, the investigators started their study by using a Vav-Cre-mediated conditional *Bmi1* depletion in all hematopoietic cells. The *Bmi1*-depleted mice exhibit decreased total cellularity in multiple hematopoietic organs, lineage cell production in peripheral blood, and blockade of B cell development. Moreover, these mice have significant decreases in multipotential progenitors (MPPs) and a decrease in HSCs during aging. Importantly, competitive bone marrow transplantation assays showed that Bmi1-deficient HSCs do not reconstitute lethally irradiated recipient mice. The above hematologic defects recapitulated observations in the Mx1-Cre-mediated *Bmi1* knockout mouse model by the investigators and others (Yu et al. 2021), suggesting a cell-intrinsic role of BMI1.

Having confirmed the impact of Bmi1 on both steady-state and stress hematopoiesis, the investigators performed singe-cell RNA-seq analysis of Bmi1-deficient HSCs. However, they did not identify any transcriptionally unique population upon Bmi1 loss despite the important role of the Bmi1-containing PRC1 complex in transcriptional regulation. Interestingly, Bmi1-deficient HSCs tend to exit the stemness state more readily than control HSCs, as they display more of a short-term HSC transcriptional signature. Next, the investigators determined whether $p16^{Ink4a}$ and $p19^{Arf}$ are the downstream mediators of Bmi1, since they are known Bmi1 targets and are among the top up-regulated hits in their RNA-seq data. However, their elegant and extensive genetic studies with various mouse models suggest that loss of either gene alone or both genes together restores Bmi1-induced hematopoietic defects, corroborating the

[*Keywords*: polycomb; self-renewal; tissue regeneration; senescence; tumor suppressor]
Corresponding authors: tongw@chop.edu, lvks@sustech.edu.cn
Article is online at http://www.genesdev.org/cgi/doi/10.1101/gad.350107.122. Freely available online through the *Genes & Development* Open Access option.

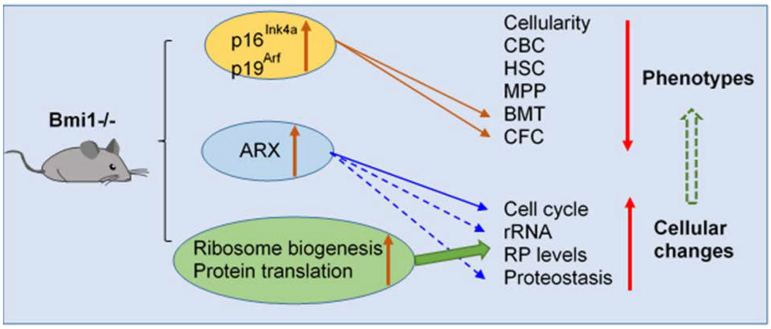

Figure 1. A schematic model summarizing how Bmi1 regulates HSC function through different mechanisms. Bmi1 deficiency leads to the increase of p16, p19, ARX, and ribosome-related genes. Loss of $p16^{Ink4a}$ and $p19^{Arf}$ only partially rescues $Bmi1^{-/-}$ HSC regeneration ability and colony-forming capacity, whereas loss of ARX partially blunts the abnormal increase of cell cycle, rRNA, and ribosome protein levels, with negligible effects on HSC function. The study identified a novel role for Bmi1 in controlling ribosome biogenesis and global protein synthesis. The resultant proteostatic stress provides a new molecular explanation of compromised HSC function and overall hematopoietic failures in *Bmi1*-deficient mice.

conclusion drawn by Oguro et al. (2006, 2010). They then switched their focus to another promising target gene, ARX, as it shows preferential expression in HSPCs of old Bmi1-deficient mice when HSCs start to be depleted. The data suggest that *ARX* loss only rescues the increased cell cycle but not other hematopoietic defects induced by Bmi1 loss, suggesting that ARX plays a minor role in this process.

Gene ontology analysis of differentially expressed genes points to several pathways related to protein synthesis. The investigators discovered a striking increase of rRNA (25 out of 87 ribosome protein-encoding genes) and, as a result, an elevation of global protein synthesis in $Bmi1^{-/-}$ HSCs. This accelerated protein synthesis adversely augments translation infidelity and protein folding burden, as $Bmi1^{-/-}$ HSPCs display more protein aggregates, a higher unfolded protein response, and more K48 polyubiquitinated proteins. These data therefore provide a direct link between Bmi1 and proteostasis that is independent of cell division. While this work provides significant insights into the contribution of current Bmi1 targets and mechanism(s) to HSC biology, it will be interesting to determine some questions in depth in the future. Does Bmi1 directly repress rRNA and RP gene expression at the transcription level? Is this repression specific to the Bmi1-containing PRC1.4 complex, instead of other PRC1 complexes? To what extent could inhibition of the protein synthesis rate (or rates) rescue the hematopoietic defects in Bmi1-deficient mice? Are there any specific genes with skewed expression at the translational level by the increased ribosome pools? Is there any correlation between Bmi1 expression and bone marrow failure syndromes clinically? The answers to these questions will facilitate our understanding toward the molecular mechanism underlying Bmi1-mediated HSC function as well as the regulation of protein synthesis rates in HSCs.

Acknowledgments

K.L. is supported by startup grant Y011286101 of the Southern University of Science and Technology. W.T. is supported by National Institutes of Health grants R01DK127738 and R01CA271523.

References

Burgess RJ, Zhao Z, Nakada D, Morrison SJ. 2022. Bmi1 suppresses protein synthesis and promotes proteostasis in hematopoietic stem cells. *Genes Dev* (this issue). doi:10.1101/gad.349917.122

Cai X, Gao L, Teng L, Ge J, Oo ZM, Kumar AR, Gilliland DG, Mason PJ, Tan K, Speck NA. 2015. Runx1 deficiency decreases ribosome biogenesis and confers stress resistance to hematopoietic stem and progenitor cells. *Cell Stem Cell* **17:** 165–177. doi:10.1016/j.stem.2015.06.002

Chua BA, Signer RAJ. 2020. Hematopoietic stem cell regulation by the proteostasis network. *Curr Opin Hematol* **27:** 254–263. doi:10.1097/MOH.0000000000000591

Geng Z, Gao Z. 2020. Mammalian PRC1 complexes: compositional complexity and diverse molecular mechanisms. *Int J Mol Sci* **21:** 8594. doi:10.3390/ijms21228594

Kang J, Brajanovski N, Chan KT, Xuan J, Pearson RB, Sanij E. 2021. Ribosomal proteins and human diseases: molecular mechanisms and targeted therapy. *Signal Transduct Target Ther* **6:** 323. doi:10.1038/s41392-021-00728-8

Kruta M, Sunshine MJ, Chua BA, Fu Y, Chawla A, Dillingham CH, Hidalgo San Jose L, De Jong B, Zhou FJ, Signer RAJ. 2021. Hsf1 promotes hematopoietic stem cell fitness and proteostasis in response to ex vivo culture stress and aging. *Cell Stem Cell* **28:** 1950–1965.e6. doi:10.1016/j.stem.2021.07.009

Lv K, Gong C, Antony C, Han X, Ren JG, Donaghy R, Cheng Y, Pellegrino S, Warren AJ, Paralkar VR, et al. 2021. Hectd1 controls hematopoietic stem cell regeneration by coordinating ribosome assembly and protein synthesis. *Cell Stem Cell* **28:** 1275–1290.e9. doi:10.1016/j.stem.2021.02.008

Nachmani D, Bothmer AH, Grisendi S, Mele A, Bothmer D, Lee JD, Monteleone E, Cheng K, Zhang Y, Bester AC, et al. 2019. Germline NPM1 mutations lead to altered rRNA 2′-O-methylation and cause dyskeratosis congenita. *Nat Genet* **51:** 1518–1529. doi:10.1038/s41588-019-0502-z

Oguro H, Iwama A, Morita Y, Kamijo T, van Lohuizen M, Nakauchi H. 2006. Differential impact of Ink4a and Arf on hematopoietic stem cells and their bone marrow microenvironment in Bmi1-deficient mice. *J Exp Med* **203:** 2247–2253. doi:10.1084/jem.20052477

Oguro H, Yuan J, Ichikawa H, Ikawa T, Yamazaki S, Kawamoto H, Nakauchi H, Iwama A. 2010. Poised lineage specification in multipotential hematopoietic stem and progenitor cells by the polycomb protein Bmi1. *Cell Stem Cell* **6:** 279–286. doi:10.1016/j.stem.2010.01.005

Park IK, Qian D, Kiel M, Becker MW, Pihalja M, Weissman IL, Morrison SJ, Clarke MF. 2003. Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature* **423:** 302–305. doi:10.1038/nature01587

Signer RA, Magee JA, Salic A, Morrison SJ. 2014. Haematopoietic stem cells require a highly regulated protein synthesis rate. *Nature* **509:** 49–54. doi:10.1038/nature13035

Tahmasebi S, Amiri M, Sonenberg N. 2019. Translational control in stem cells. *Front Genet* **9:** 709. doi:10.3389/fgene.2018.00709

Yu H, Gao R, Chen S, Liu X, Wang Q, Cai W, Vemula S, Fahey AC, Henley D, Kobayashi M, et al. 2021. Bmi1 regulates Wnt signaling in hematopoietic stem and progenitor cells. *Stem Cell Rev Rep* **17:** 2304–2313. doi:10.1007/s12015-021-10253-4