Hematopoietic stem cell fate under the influence of Ser/Thr protein phosphatases

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Received: February 22, 2024.
Accepted: February 27, 2024.
Early view: March 7, 2024.

https://doi.org/10.3324/haematol.2024.285040

Hematopoietic stem cells (HSC) are multipotent cells capable of unlimited self-renewal and are essential for production of blood and immune cells throughout life. HSC reside in a quiescent state in the bone marrow and proliferate only after certain stimuli. Failure in wakening these quiescent cells may result in hematologic defects, and, therefore, this process is tightly regulated by multiple signaling pathways. Recent research suggests that Ser/Thr protein phosphatases may be involved in HSC biology more than previously anticipated.

In this issue, Lu and colleagues show that protein phosphatase PPM1B controls homeostasis of HSC through regulation of the Wnt/β-catenin signaling pathway. Using a transgenic *Ppm1b*^{CKO} mouse model with VAV-Cre mediated conditional deletion of exon 2 of the *Ppm1b* gene in hematopoietic cells, they showed that PPM1B is necessary for proliferation of HSC.[1] Impaired functionality of HSC in *Ppm1b*^{CKO} animals was further demonstrated by limiting dilution assays and serial transplantation experiments. Data from the animal model were recapitulated *in vitro* using the small molecule inhibitor of PPM1B (HN252[2]), as well as by depletion of PPM1B by RNA interference. In addition, *Ppm1b*^{CKO} mice also exhibited alterations in common lymphoid progenitors, which resulted in B-cell leukocytopenia, whereas the myeloid lineage was unaffected. Furthermore, RNAseq analysis from Lineage$^-$ Sca-1$^+$ c-Kit$^+$ (LSK) hematopoietic stem and progenitor cells revealed that several signaling pathways, including WNT, were dysregulated in *Ppm1b*^{CKO} animals. In particular, several downstream targets of β-catenin, including *Fzd1*, *Jun*, *Camk2b*, *Lrp5*, *Ccnd1* and *Gpc4*, were down-regulated upon the deletion of *Ppm1b* suggesting that the defect in HSC may be caused by suppression of WNT signaling. Indeed, LSK cells from *Ppm1b*^{CKO} animals showed increased levels of the inactive form of β-catenin, which is phosphorylated at Ser33/37/Thr41. Finally, the Authors nicely demonstrated that stimulation of the WNT pathway by BML-284 rescued the phenotypes in *Ppm1b*^{CKO} mice, supporting the conclusion that PPM1B controls HSC through stimulation of the WNT pathway.

PPM1B belongs to a conserved PP2C family of Ser/Thr phosphatases that require binding of manganese/magnesium ions for their activity. They function as single subunit enzymes and their substrate specificity is influenced by internal linker regions. Interestingly, other members of this conserved phosphatase family have also been implicated in hematopoiesis. In particular, mitochondrial PPM1K was proposed to regulate branched amino acid catabolism in HSC and loss of PPM1K impaired maintenance of the HSC pool.[3] In addition, loss of the nuclear PPM1D (also called WIP1) phosphatase caused mTORC-dependent expansion of the HSC compartment in *Ppm1d*$^{-/-}$ animals, whereas truncating gain-of-function mutations in *PPM1D* reduced self-renewal of HSC.[4,5] Interestingly, the truncated PPM1D stimulates HSC survival after genotoxic stress by inhibiting the p53 pathway and can promote therapy-induced acute myeloid leukemia.[5,6] Altogether, these observations point at the crucial role of Ser/Thr phosphatases in the regulation of HSC properties and their potential contribution to the development of hematologic disorders. In fact, several phosphatases have been implicated in hematologic malignancies as well as in solid tumors, and may represent attractive pharmacological targets in future clinical interventions. The development of small molecule inhibitors to protein phosphatases is challenging, and few compounds show satisfactory specificity and efficiency in cellular and animal models. For example, the selective PPM1D inhibitor GSK2830371 suppressed the growth of p53-positive cancer cells *in vitro* and in animal models.[5-7] In the present study, Lu and colleagues used a new PPM1B inhibitor (HN252), proved its efficiency in animal models, and elegantly validated its specificity in the *Ppm1b*^{CKO} animals.[1,2] This tool will be invaluable for exploring the functions of PPM1B in several systems. Additionally, consid-

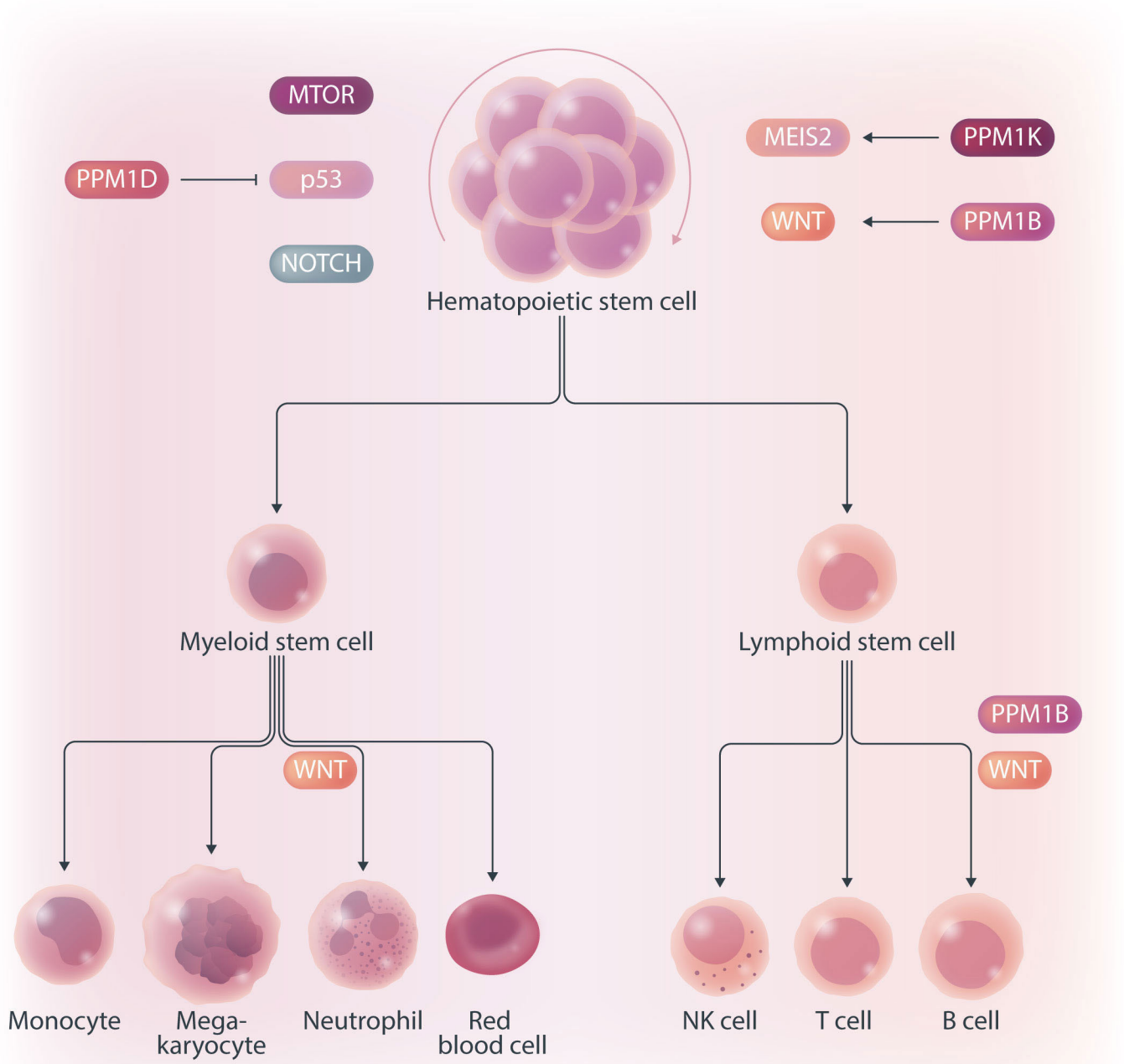

Figure 1. PP2C family protein phosphatases regulating the hematopoiesis. PPM1B and PPM1K promote self-renewal of hematopoietic stem cells (HSC) through activating WNT/b-catenin and MEIS2 pathways, respectively. In contrast, PPM1D promotes differentiation of HSC by modulating MTOR, p53 and NOTCH pathways. In addition, PPM1B regulates production of B cells but, surprisingly, is not needed in myeloid lineage.

ering the B-cell leukocytopenia observed in *Ppm1b*^{CKO} mice, it might be interesting to investigate the potential of PPM1B inhibitor in suppressing B-cell leukemias.

Mechanistically, Lu and colleagues point at deregulation of the Wnt/β-catenin signaling pathway as the underlying cause of the B-cell phenotype present in *Ppm1b*^{CKO} mice. Nevertheless, the role of the Wnt/β-catenin signaling pathway in the hematopoietic system is controversial, and discrepancies are justified by the use of different models and approaches.[8] Here, Lu and colleagues bring into the field a new player, PPM1B, which by its dephosphorylating action can promote the active form of β-catenin, and thus enhance the activity of this pathway. However, while β-catenin is critical for T-cell,[9] B-cell,[10] and granulocytic development,[11,12] they report alterations only in the B-cell compartment. Thus, we can hypothesize that PPM1B does not act in myeloid progenitors or T cells, or that it does not regulate β-catenin phosphorylation uniformly in all hematopoietic cells. Ultimately, since the Wnt/β-catenin signaling pathway is regulated at multiple levels and is subjected to spatiotemporal regulations, in the future it will be interesting to investigate the impact of PPM1B on this signaling pathway also in other tissues besides the bone marrow.

In summary, Ser/Thr protein phosphatases of the PP2C family are now emerging as new regulators of hematopoiesis and potential pharmacological targets. Their possible clinical use will depend on the development and careful validation of selective small molecule inhibitors.

Disclosures

No conflicts of interest to disclose.

Contributions

LM wrote the manuscript with the input from MAJ.

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