Comment on Greenberg et al, page 1180

CD53 sends HSCs to sweet DREAMs

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In this issue of *Blood*, Greenberg et al[1] show that hematopoietic stem cells (HSCs) employ a novel mechanism via promotion of the CD53-mediated dimerization partner, Rb-like, E2F and multi-vulval class B (DREAM) complex to return to quiescence after inflammatory and proliferative stress, thereby protecting them from functional decline.

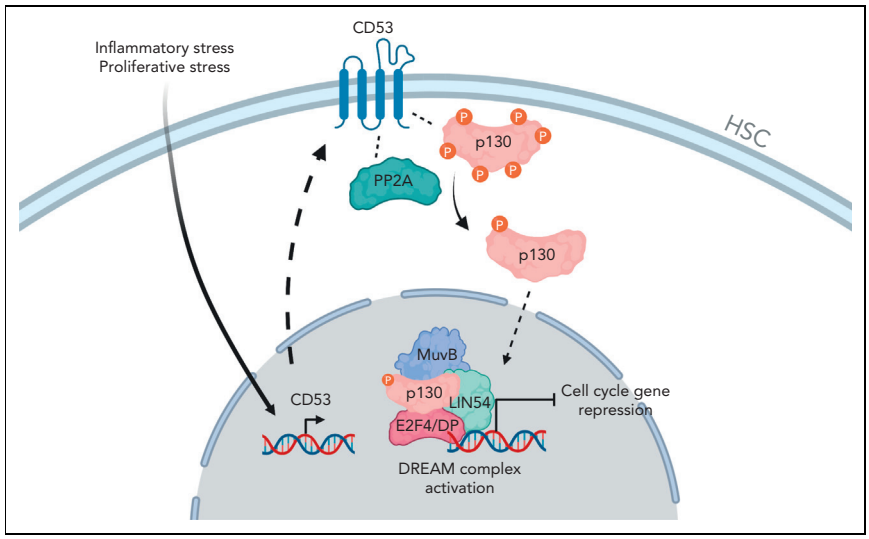
HSCs are a population of rare cells that are capable of replenishing the whole blood system. To preserve this lifelong ability, HSCs are maintained in a quiescent and dormant state under homeostatic conditions.[2,3] Under stress conditions, such as viral infections, HSCs are forced to exit their quiescent state and enter the cell cycle. However, little is known about the mechanisms regulating the return of HSCs to quiescence, which is a crucial step to prevent both stem cell exhaustion and the development of hematopoietic malignancies.

CD53 is a member of the tetraspanin family of transmembrane proteins that regulate cellular processes, such as migration, adhesion, and signaling.[4] CD53 has been previously identified as an asymmetrically segregating protein in HSCs and is enriched in more stem-like HSCs.[5] In addition, the authors have recently shown that CD53 regulates early B-cell development by promoting interleukin 7 receptor.[6] However, the mechanism by which CD53 regulates HSC function remains unclear.

In this study, Greenberg et al show that CD53 is transiently upregulated in both mouse and human HSCs on inflammatory and proliferative stress treatments.[1] Under homeostatic conditions, no differences were found in the frequency or absolute number of HSCs in the bone marrow or spleen between wild-type mice and mice lacking CD53. However, on treatment with the stem cell mobilization agent granulocyte colony-stimulating factor (G-CSF), loss of CD53 resulted in prolonged cycling, reduced HSC function under stress conditions, and return to quiescence.

To understand the mechanism by which CD53 protects HSC function in response to stress, the authors performed transcriptomic analysis of CD53 knockout and wild-type HSCs on G-CSF treatment. This analysis revealed differential expression of DREAM complex target genes in CD53 knockout HSCs, in line with the observed dysregulation of cycling. The DREAM complex has been shown to be a master coordinator of cell cycle–dependent gene expression,[7] but little was known about its role in HSCs. Strikingly, inhibition of the cell cycle with the cyclin-dependent kinase 4/6 (CDK4/6) inhibitor palbociclib equalized the repopulating activity of CD53 knockout HSCs and the expression of DREAM complex target genes, indicating that CD53 largely acts on HSC cell cycle regulation.

It is known that palbociclib inhibits CDK4/6, which prevents phosphorylation of the riboblastoma-like proteins p107 and p130, thereby promoting the DREAM complex activity and thus inhibiting the cell cycle. In this study, the authors show that p107 and p130 exhibited increased phosphorylation levels in CD53 knockout HSCs exposed to proliferative stress. The authors next hypothesize that CD53 regulates the levels of the pocket proteins p107 and p130, consequently promoting their active, hypophosphorylated form and their binding to the DREAM complex. They confirm this hypothesis by proximity ligation assays, first showing that CD53 interacts individually with both p130 and its phosphatase PP2A; and, second, by demonstrating that p130 and PP2A only interact in the presence of CD53. Taken together, these results suggest that CD53 prevents p130 degradation by promoting its PP2A-mediated hypophosphorylation and thereby enhancing the activity of the DREAM complex (see figure).



Tetraspanin protein CD53 is transiently upregulated in HSCs on stress. CD53 localizes closely to p130 and its hydratase PP2A, promoting the hypophosphorylated and active p130 form. Once dephosphorylated, p130 relocates to the nucleus and enhances DREAM complex formation, activation, and transcriptional repression of cell cycle genes, thereby promoting HSC return to quiescence. MuvB, multi-vulval class B; LIN54, Lin-54 DREAM MuvB core complex component; DP, dimerization partner. Figure created with BioRender.com.

Tetraspanin proteins, such as CD63, CD81, and CD82, have been shown to regulate HSC quiescence through the transforming growth factor-β and protein kinase B (Akt) signaling pathways.[8-10] This work highlights a novel protection mechanism mediated by the tetraspanin CD53. CD53 preserves HSC function on inflammatory and proliferative stress by promoting the activity of the DREAM complex and the return of HSCs to a quiescent state. It will now be of great interest to address the role of this axis in conditions in which the HSC pool expands but loses its self-renewal capacity, such as in aging. Future studies should identify direct and indirect targets of these interactions and investigate their functional role in human HSCs to (1) expand the understanding of how HSC quiescence is regulated and (2) identify potential novel targets to treat hematopoietic malignancies.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

REFERENCES

1. Greenberg ZJ, Monlish D, Chiquetto Paracatu L, et al. The tetraspanin CD53 protects stressed hematopoietic stem cells via promotion of DREAM complex–mediated quiescence. *Blood*. 2023;141(10):1180-1193.
2. Cabezas-Wallscheid N, Buettner F, Sommerkamp P, et al. Vitamin A-retinoic acid signaling regulates hematopoietic stem cell dormancy. *Cell*. 2017;169(5):807-823.e19.
3. Wilson A, Laurenti E, Oser G, et al. Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. *Cell*. 2008;135(6):1118-1129.
4. Hemler ME. Tetraspanin functions and associated microdomains. *Nat Rev Mol Cell Biol*. 2005;6(10):801-811.
5. Beckmann J, Scheitza S, Wernet P, Fischer JC, Giebel B. Asymmetric cell division within the human hematopoietic stem and progenitor cell compartment: identification of asymmetrically segregating proteins. *Blood*. 2007;109(12):5494-5501.
6. Greenberg ZJ, Monlish DA, Bartnett RL, et al. The tetraspanin CD53 regulates early B cell development by promoting IL-7R signaling. *J Immunol*. 2020;204(1):58-67.
7. Sadasivam S, DeCaprio JA. The DREAM complex: master coordinator of cell cycle-dependent gene expression. *Nat Rev Cancer*. 2013;13(8):585-595.
8. Lin KK, Rossi L, Boles NC, Hall BE, George TC, Goodell MA. CD81 is essential for the re-entry of hematopoietic stem cells to quiescence following stress-induced proliferation via deactivation of the Akt pathway. *PLoS Biol*. 2011;9(9):e1001148.
9. Saito-Reis CA, Marjon KD, Pascetti EM, Floren M, Gillette JM. The tetraspanin CD82 regulates bone marrow homing and engraftment of hematopoietic stem and progenitor cells. *Mol Biol Cell*. 2018;29(24):2946-2958.
10. Hur J, Choi JI, Lee H, et al. CD82/KAI1 maintains the dormancy of long-term hematopoietic stem cells through interaction with DARC-expressing macrophages. *Cell Stem Cell*. 2016;18(4):508-521.

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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Mo et al, page 1194

Harnessing ADR T cells to enhance allo-HCT

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In this issue of *Blood*, Mo et al[1] show that human T cells engineered to express an OX40 cytotoxic receptor and a CD19-targeted chimeric antigen receptor (CAR) protect animals from fatal xenogeneic graft-versus-host disease (GVHD) and leukemia relapse following allogeneic hematopoietic cell transplantation (allo-HCT).

Allo-HCT is a potentially curative treatment for patients with hematologic malignancies. Yet, morbidity and mortality due to relapse, GVHD, and infectious complications limit its therapeutic efficacy. The current approaches to decrease acute GVHD, including administering immunosuppressive medications,[2] depleting T cells from the allograft before transplantation,[3] and administering posttransplant cyclophosphamide for in vivo depletion of T cells,[4] are associated with either increased infectious complications or relapse. Hence, a central area of ongoing investigation in the field of allo-HCT focuses on strategies to enhance graft-versus-leukemia activity while limiting GVHD.

Prior data demonstrated the upregulation of a costimulatory receptor, OX40, on activated CD4 T cells and a subset of CD8 T cells.[5,6] OX40 has also been found to mediate expansion of alloreactive T cells. Further work from several groups elucidated that the selective depletion of OX40$^+$ alloreactive cells from the allograft or OX40 blockade decreased the expansion of alloreactive T cells while preserving antitumor and antiviral activity.

Thus, Mo et al sought to advance the targeting of OX40 on activated T cells as a strategy to decrease GVHD without compromising beneficial T-cell activity. First, the authors found that OX40 was upregulated on T cells that infiltrated GVHD target organs in a rhesus macaque allo-HCT model. Notably, previous data on OX40$^+$ T cells in tissues damaged by acute GVHD were limited. Next, the authors developed a cytotoxic OX40-specific alloimmune defense receptor (OX40.ADR), consisting of an extracellular domain of OX40 ligand with 4-1BB and CD3ζ signaling domains. Human T cells were retrovirally transduced to express the OX40.ADR, and the authors found that the ADR T cells suppressed alloreactivity in vitro. In a xenogeneic mouse allo-HCT model, donor ADR human T cells protected mice from fatal xenogeneic GVHD. The authors also showed that targeting OX40 with ADR did not limit the antiviral activity of T cells in vitro or in vivo. Furthermore, CAR-ADR T cells induced leukemia clearance and decreased GVHD in tumor-bearing mice in a xenograft model of residual disease post-transplantat. The authors' findings underscore a potential approach to engineer T cells from donor sources to decrease GVHD and relapse following allo-HCT.

Prior work by the authors[7] as well as other investigators has evaluated the use of donor adoptive cell therapies that target antigens, such as 4-1BB[7] and CD83. These markers are expressed on alloreactive T cells, and depleting them in the