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Blood 142 (2023) 5602

The 65th ASH Annual Meeting Abstracts

ONLINE PUBLICATION ONLY

503.CLONAL HEMATOPOIESIS, AGING AND INFLAMMATION

The Tetraspanin CD53 Promotes the Clonal Advantage of Dnmt3a-Deficient Hematopoietic Stem Cells

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Clonal hematopoiesis involves the selective expansion of HSCs with mutations, most commonly in genes such as the epigenetic modifiers *TET2* and *DNMT3A*, and confers an increased risk of hematopoietic malignancies, cardiovascular disease and all-cause mortality. Studies show that *Tet2*- or *Dnmt3a*-mutant HSCs expand relative to their WT counterparts in response to inflammatory stress, indicating that under these conditions, mutant HSCs have a resistance to inflammatory cytokines and selective advantage. It has been reported, and confirmed in our lab with confocal microscopy on purified HSCs, that expression of the tetraspanin CD53 is markedly increased in HSCs lacking *Dnmt3a* or *Tet2* compared to WT. While normally expressed at low levels, WT HSCs transiently upregulate CD53 in response to inflammatory stimuli. We recently reported that this transient elevation in CD53 expression protects the function of stressed HSCs by promoting their return to quiescence. Given this role of CD53, we hypothesize that the elevated CD53 in mutant HSCs contributes to their clonal advantage.

To test the possible role of CD53 in the expansion of mutant HSCs, we first used lentivirus to enforce expression in WT HSCs and tag them with GFP. Lethally irradiated recipient mice were transplanted with minority populations of sorted CD53-overexpressing HSPCs together with majority populations of WT competitor bone marrow. These experiments revealed that HSCs overexpressing CD53 had superior multilineage engraftment to empty vector control HSCs in primary competitive transplants (10% vs 0.2% GFP+ at 12 weeks). Consistently, serial re-plating assays show that CD53-overexpressing HSCs form a greater number of colonies week to week in Methocult.

Next, to address the function of CD53 in mutant HSCs, we assessed HSC function in Dnmt3aflx/flx;Vav-iCre and Dnmt3aflx/flx;Vav-iCre;Cd53-/- mice. HSC frequencies at baseline were comparable between groups, and they engrafted similarly in primary competitive transplants into lethally irradiated recipient mice. However, in secondary transplants, Dnmt3aflx/flx;Vav-iCre;Cd53- /- HSCs exhibited lower overall engraftment compared to Dnmt3aflx/flx;Vav-iCre HSCs (46% vs. 73% chimerism at 16 weeks post-transplant). In support of this finding, Dnmt3aflx/flx;Vav- iCre;Cd53-/- HSCs showed mitigated serial replating capacity in Methocult (2 vs 5+ re-platings).

To evaluate CD53's role in mutant HSC clonal expansion in response to inflammatory stress, we generated bone marrow chimeras with either Dnmt3aflx/flx;Vav-iCre or Dnmt3aflx/flx;Vav-iCre;Cd53-/- HSCs, along with WT HSCs, and treated them with IFNg daily for 30 days. While Dnmt3aflx/flx;Vav-iCre HSCs expanded with treatment over WT HSCs, Dnmt3aflx/flx;Vav-iCre;Cd53-/- HSCs did not. Together, these data indicate a critical role for CD53 in promoting mutant HSC clonal expansion. Similar studies with Tet2-mutant mice are ongoing, and other studies underway aim to understand the mechanism by which elevated CD53 supports mutant HSCs.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-180980