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Blood 142 (2023) 2694

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

503.CLONAL HEMATOPOIESIS, AGING AND INFLAMMATION

Id1 Ablation Rescues Clonal Hematopoiesis and Delays Disease Progression in *Tet2* Null Mice

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Hematopoietic malignancies are thought to emerge through the gradual acquisition of genetic mutations within hematopoietic stem and progenitor cells (HSPCs). Some mutations impart a selective proliferative/growth advantage to HSPCs, which expand over time and contribute to a substantial percentage of mature blood cells. This increased proliferation and expansion is termed clonal hematopoiesis (CH) and is a preleukemic phase that is associated with an increased risk of accumulating additional mutations and developing leukemia. These mutations are also associated with increased inflammatory stress and increased cytokine production ("cytokine storm") in the hematopoietic microenvironment which can contribute to clonal hematopoiesis.

Inhibitor of DNA binding 1 (ID1) protein is induced by hematopoietic growth factors and other mediators of inflammatory stress and promotes HSPC proliferation by regulating E proteins and their targets. HSCs that lack *Id1* are protected from chronic proliferative stress and exhaustion during serial BMT, and other chronic physiologic stress including genotoxic and inflammatory stress, and aging. ID1 is frequently overexpressed in hematopoietic malignancies and enforced expression of *Id1* in HSPCs promotes a myeloproliferative disease in mice suggesting that ID1 may drive clonal expansion and regulate the growth of some hematologic malignancies. Mutations in the Ten-Eleven-Translocation-2 (*Tet2*) (Tet methylcytosine dioxygenase 2) gene are frequently observed in patients with CH, and mice that lack Tet2 show clonal expansion, increased mutational loads, genomic instability that results in myeloid and lymphoid malignancies after a long latency. Here, we show that ablation of *Id1* rescues clonal hematopoiesis and delays disease progression in *Tet2* $^{-/-}$ mice.

We found that *Tet2* $^{-/-}$ HSPCs express high levels of *Id1*. Therefore, we genetically ablated *Id1* in *Tet2* $^{-/-}$ mice and generated *Tet2* $^{-/-}$;*Id1* $^{-/-}$ (dKO) mice and performed competitive bone marrow transplantation studies (BMT) to study the intrinsic role of these genes in hematopoietic development. We found that genetic ablation of *Id1* in *Tet2* $^{-/-}$ HSPCs reduces HSPC proliferation/expansion and myeloid skewing by flow cytometry. dKO mice showed reduced spleen size and weight indicating that reducing ID1 levels in *Tet2* $^{-/-}$ mice rescues extramedullary hematopoiesis. We confirmed that reducing ID1 levels in *Tet2* $^{-/-}$ mice reduces clonal expansion of *Tet2* $^{-/-}$ BMCs using limited number of donor BMCs in competitive BMT. Furthermore, we performed functional assays including colony forming assays (CFU-C) and single cell (SC) assays to assess the proliferation of these cells and found that loss of *Id1* in *Tet2* $^{-/-}$ mice reduced 1) the number of CFU-C's and the serial replating ability of *Tet2* $^{-/-}$ HSPCs, and 2) progenitor expansion in SC assays.

Moreover, dKO recipient mice BMCs showed increased apoptosis and senescence compared to *Tet2* $^{-/-}$BMCs, which could contribute to reduced expansion. Surprisingly, *Id1* ablation in *Tet2* $^{-/-}$ mice also resulted in reduced number of chromosomal aberrations indicating that reducing ID1 levels in *Tet2* $^{-/-}$ mice rescues genomic instability. Furthermore, dKO recipient mice showed delayed onset of disease in a model of chronic proliferative stress. Mechanistically, we found that p16 expression was significantly increased in the dKO recipient mice, which could account for reduced expansion and increased apoptosis and senescence. shRNA mediated knockdown of p16 in dKO lineage negative cells reduced apoptosis and senescence in these cells confirming the role of p16 in these phenotypes. We also found that 53BP1 and Rad51 expression was reduced in the dKO recipient mice, which could lead to improved DNA repair and genomic stability. Thus, Id1 may represent a potential therapeutic target to reduce clonal expansion and delay the onset of leukemia.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-186744

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