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https://doi.org/10.1182/blood.2022018005

HEMATOPOIESIS AND STEM CELLS

Comment on Li et al, page 1686

Forever young: Sphk2 in HSCs, when less is more

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In this issue of *Blood*, Li et al[1] use different mouse models to comprehensively dissect the role of sphingosine kinases (Sphks) in the regulation of hematopoietic stem cell (HSC) function, uncovering that specific loss of Sphk2 expands and functionally rejuvenates HSCs.

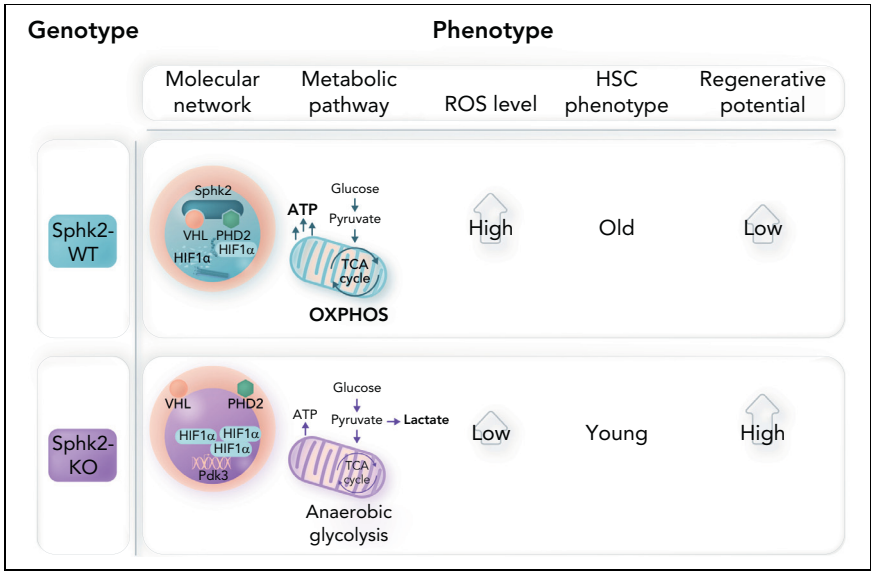
HSCs sustain the lifelong production of most adult blood and immune cell lineages. At the apex of the hematopoietic system, HSCs are characterized by their capacity for long-term self-renewal and their ability to differentiate into mature cells.[2] The decline in their regenerative potential is a hallmark of aging, which contributes to the progressive physiological dysfunction and increased risk for age-dependent disorders.[3]

Multiple studies have tried to elucidate potential mechanisms of HSC aging. In this context, heterochronic parabiosis (a rejuvenating intervention where circulatory systems of aged and young mice are surgically connected) can partially reverse some of these phenotypes. However, whether HSCs are responsive/refractory to this approach remains controversial.[4,5] Moreover, the detailed mechanisms mediating its effects are not fully elucidated. Thus, understanding how/why HSCs age is critical to design interventions to prevent, delay, alleviate, or reverse the physiological consequences of aging.

Sphks exist as 2 different isoforms in mammals (Sphk1 and Sphk2), which synthesize sphingosine 1-phosphate (S1P), a bioactive lipid molecule that regulates multiple processes.[6] Genetic abrogation of both kinases is embryonically lethal; however, *Sphk1*-knockout (KO) and *Sphk2*-KO mice are viable and healthy, suggesting functional redundancy between both.[6] In this study, Li and colleagues used a variety of in vitro and in vivo experimental assays to uncover that loss of Sphk2 in the hematopoietic compartment (but not Sphk1) results in expansion of quiescent HSCs without affecting other mature blood cell lineages. Interestingly, loss of Sphk2 is not compensated by increased Sphk1 levels. In addition, even if Sphk2 loss results in decreased S1P levels, the *Spkh2*-KO phenotype is S1P independent, suggesting that Sphk2 catalytic activity is dispensable for these effects.

To assess the long-term functional relevance of Sphk2 deficiency, the authors performed a series of rigorous reconstitution experiments in vivo. Their results demonstrated that *Sphk2*-KO bone marrow cells have enhanced self-renewal potential and increased reconstitution capabilities in a cell-autonomous manner, without compromising linage commitment. Even in conditions that force HSC proliferation and differentiation, such as 5-fluorouracil treatment or lethal irradiation, *Sphk2*-KO mice show significant survival extension, and *Sphk2*-KO HSCs preserve an improved regenerative potential. This is highly relevant because hematological toxicity is one of the main causes for chemotherapy discontinuation in cancer patients. Thus, small molecules pharmacologically targeting Sphk2 might help prevent/treat these adverse events.

The authors further investigated the role of Sphk2 in HSC aging by integrating computational approaches with additional reconstitution assays comparing HSCs from aged and young mice. Consistent with previous results, Sphk2 deficiency prevents the acquisition of age-induced transcriptional signatures in old HSCs, such that *Sphk2*-KO old HSCs present phenotypic and functional characteristics like those observed in young wild-type HSCs. In line with this, old HSCs show higher Sphk2 levels than young HSCs. Functional annotation of genes downregulated in *Sphk2*-KO HSCs suggested reduced oxidative phosphorylation (OXPHOS). Conversely, upregulated genes were involved in the regulation of anaerobic glycolysis. In this context, HSCs are extremely



In hypoxic environments, loss of Sphk2 increases PDK3 expression via HIF1α stabilization, promoting anaerobic glycolysis, reducing ROS levels, and increasing the regenerative potential of more youthful HSCs. WT, wild-type. Professional illustration by Somersault18:24.

sensitive to reactive oxygen species (ROS) levels.[7] Indeed, *Sphk2*-KO HSCs show increased lactate dehydrogenase activity (and lactate production) and present lower oxygen consumption rates ex vivo, with reductions in both adenosine triphosphate and ROS levels (see figure). It is well known that, in hypoxic environments, quiescent HSCs have low energy demands and mainly rely on anaerobic glycolysis; however, upon activation, they rapidly switch to OXPHOS to satisfy the metabolic requirements of differentiation.[7] Li et al's findings suggest that Sphk2 loss in HSCs might interfere with this metabolic shift, allowing a younger metabolic state that preserves stemness and enhances their regenerative capacity. Interestingly, *Sphk2*-KO HSCs showed decreased glucose uptake even if increased lactate levels suggest a more glycolytic phenotype. Thus, metabolic tracing with ^{13}C-glucose to experimentally confirm and expand on these apparently conflictive findings would be needed. Current technical limitations prevent from easily using this technique in HSCs; however, recent advances in single-cell and imaging metabolomic approaches could help address this issue in the short-term. Moreover, HSCs show higher Sphk2 levels compared with differentiated cells, suggesting that HSCs need to be metabolically primed to rapidly respond to activation signals and/or that maintenance of the tricarboxylic acid (TCA) cycle in anaerobic conditions is required to provide essential intermediates for the epigenetic regulation of stemness.

Finally, the authors identified HIF1α as a downstream target of Sphk2, consistent with the well-known effects of HIF1α in HSCs.[8] Robust in vitro experiments in combination with computational modeling demonstrated that Sphk2 physically interacts with PHD2 and VHL in the HSC nucleus to regulate HIF1α stability in a proteasome-dependent manner (again independently from Sphk2 catalytic activity). Indeed, loss of VHL in hematopoietic progenitors significantly reduced their mitochondrial content,[9] suggesting a similar glycolytic phenotype to the one in *Sphk2*-KO cells.

HIF1α is a key activator of glycolysis in resting HSCs, where it senses low oxygen levels and activates PDK2/PDK4 to prevent pyruvate entry into the TCA cycle by inhibiting pyruvate dehydrogenase (PDH).[9] In the context of Sphk2 loss, there is selective upregulation of PDK3 that leads to strongly decreased PDH activity (see figure). Importantly, genetic reduction of either *Hif1α* or *Pdk3* partially rescues the effects of Sphk2 loss by promoting an OXPHOS switch.

Still, Sphk2 has broad substrate specificity;[6] thus, the potential implication of other Sphk2-phosphorylated targets requires additional investigation. Similarly, further research is warranted to determine how cell-intrinsic Sphk2 loss creates a chromatin-permissive environment for HIF1α to regulate the expression of different PDK enzymes. Finally, considering the known heterogeneity of HSCs,[2] it is tempting to speculate that high Sphk2 might be a marker of functionally old HSCs. Additional single-cell studies should address this question.

Overall, Li et al unveiled a novel Sphk2-HIF1α-PDK3 axis as a checkpoint in the control of HSC metabolic fitness. This study represents a milestone in our understanding HSC metabolism/aging, adds Sphk2 to a growing list of master regulators of HSC functions,[10] and sets the stage for future HSC rejuvenation strategies via boosting PDK3 expression, enhancing HIF1α stability, and/or targeting Sphk2.

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

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https://doi.org/10.1182/blood.2022017932

LYMPHOID NEOPLASIA

Comment on Slager et al, page 1702

The clonal evolution and natural history of MBL

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In this issue of Blood, Slager et al[1] describe the results of large-scale screening of more than 10 000 individuals from the Mayo Clinic Biobank for a monoclonal B-cell lymphocytosis (MBL). Eight-color flow cytometry was used with a sensitivity of 0.005% for the detection of 20 or more monoclonal B-cell events. The 3 previously defined immunophenotypes of $CD5^+$ chronic lymphocytic leukemia (CLL)-like MBL, atypical MBL (bright CD20), and $CD5^-$ MBL remain unchanged. The definitions of low-count MBL (LC-MBL; <500 cells per μL) and high-count MBL (HC-MBL; >500 cells per μL and <5000 cells per μL) are based on the absolute B-cell count. CLL is defined as an absolute B-cell count of >5000 cells per μL. In the absence of a complete blood count white blood cell and the absolute lymphocyte count, the estimated clone size can be used to designate LC-MBL (<15%) and HC-MBL (>85%). One final comment about nomenclature: LC-MBL sometimes is referred to as "population-based MBL," whereas HC-MBL is referred to as "clinical MBL."

Several findings emerge from this study that are confirmatory or constitute new findings that increase our understanding of MBL not only as a precursor to CLL, but also as a prognostic biomarker. First, the prevalence of MBL is 17%, of which 95% is LC-MBL. The pattern of increasing MBL with age and the male-to-female ratio was confirmed. The clone size based on the percentage of clonal B cells of the 3 immunophenotypes was 1.9%, 13%, and 28.2% for CLL-like MBL, atypical MBL, and $CD5^-$ MBL, respectively. Shim et al[2] reported a similar pattern in MBL blood bank donors. The presence of more than one clone has been reported in individuals with MBL as well as in those with CLL. The clinical significance of this finding is not known. A 4.3-fold increased risk of lymphoid malignancy is present in LC-MBL and a 75-fold increased risk is present in HC-MBL.

Slager et al[1] note that in many cases, the lymphoid neoplasms that arise in the setting of MBL have an immunophenotype distinct from the pre-existing MBL clone. They further suggest "that it may represent origination of an independent clonal process rather than "progression" of the LC-MBL clone." An early report by Tembhare et al[3] supports this idea. Given the high prevalence of LC-MBL, more examples as described by Slager et al[1] are likely to be seen if they are sought at the diagnosis of other lymphoid neoplasms. An oligoclonal origin of MBL has been suggested.[4] That the great majority of LC-MBL cases do not progress to CLL, but rather remain stable, has given rise to the idea of an aging immune system or immuno-senescence.[5] Also, sporadic MBL is different from familial MBL.

Our understanding of the natural history of MBL depends on biorepositories, particularly those with 2 or more time points of stored samples before the diagnosis of CLL. This allows not only an estimate of prevalence, but also ascertainment of whether the MBL is stable, progressive, or transient. The striking finding in all these studies is the number of years that MBL may precede the diagnosis of CLL: literally decades in some cases.[6] Further, it seems that this is independent of immunoglobulin gene heavy chain variable region mutational status and perhaps even fluorescence in situ hybridization results.

What about familial CLL and MBL? Mayo Clinic investigators and their National Cancer Institute colleagues have carried out a similar study screening for the prevalence and observing the natural history of MBL among first-degree relatives in several CLL kindreds.[7] Of course, the prevalence is much higher. At baseline, the prevalence of MBL was 22% and the progression from healthy to LC-MBL to HC-MBL to CLL was documented at a 1.1% annual rate. The unaffected first-degree relatives provide an opportunity to investigate further the natural history of MBL. It would be of interest to learn more about the B-cell repertoire before and after the onset of MBL. Is it related to infection? What are the steps leading to MBL? Preunmutated MBL? Premutated MBL? Pre-13q14 del MBL? Pretrisomy 12 MBL? Many molecular findings in MBL also are found in early CLL.[8] The number of susceptibility loci (single nucleotide polymorphisms [SNPs]) associated with CLL continue to increase. The number now stands at 41 SNPs observed in CLL among patients with CLL of European ancestry.[9] A polygenic risk score (PRS) based on a weighted combination of individual loci linked to the risk of CLL PRS was developed. Using cryopreserved samples from the Mayo Clinic CLL Resource and Biobank, Kleinstern et al[9] extended these studies to MBL, which seems to share approximately 50% of known CLL SNPs. More remarkable is that the progression of the PRS reported in this study increased from healthy to LC-MBL to HC-MBL to CLL (see Figure 1A in Kleinstern et al[9]). The authors suggest that "these loci may be associated with progression from MBL to CLL rather than associated with initiation of the B-cell clone."

The study of familial CLL pedigrees adds another layer of complexity to the germline origin of MBL. Jønsson et al[10] recently compared Danish and Norwegian families with unrelated parents and the inbred Faroese