Blood aging

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Is the philosopher's stone to rejuvenate blood stem cells an epigenetic regulator?

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Aging is associated with an accumulation of myeloid-biased hematopoietic stem cells with reduced regenerative potential, but the underlying mechanisms remain unclear. A study by Wendorff et al. demonstrates that inactivation of a single epigenetic regulator – the plant homeodomain factor 6 (PHF6) – transcriptionally and functionally rejuvenates mouse aged hematopoietic stem cells.

Things change as we age. Older adults, as we have witnessed during the COVID-19 pandemic, have a higher risk of infections and anemia, causing fatigue[1,2]. In addition, leukemia becomes much more prevalent during aging. Collectively, an age-associated failure of blood cell production contributes to morbidity and mortality. These age-related changes can be traced back to the origin of the blood system – that is, to the rare hematopoietic stem cells (HSCs) that lodge in the bone marrow. The holy grail of regenerative medicine is to identify the cellular and molecular aspects that go astray with aging and then reverse these to a youthful state, such that homeostatic blood cell production is maintained during the lifespan of an individual.

Despite substantial progress and the identification of multiple molecular mechanisms that contribute to the demise of aged HSCs, it has been difficult to restore young-like functions in aged HSCs. In a study published in this issue of *Nature Aging*, Wendorff and colleagues bring a new fresh breeze into this long-standing puzzle and place epigenetic programming – a somewhat forgotten aspect of aging in the HSC field – into the spotlight[3]. Remarkably, by suppressing a single epigenetic regulator (PHF6) the authors were able to prevent most of the functional decline of HSCs and, more surprisingly, rejuvenate the hematopoietic system in aged mice.

Paradoxically, aged HSCs show a substantial expansion in numbers but are much less potent. Aged HSCs produce an excess of myeloid cell types at the expense of lymphoid lineages (a phenomenon known as myeloid skewing), which affects immunity and leukemia development[4]. *PHF6* is commonly mutated and implicated in leukemia progression[5]. Interestingly, together with other epigenetic regulators such as DNMT3A, TET2 and AXL1, PHF6 is also commonly altered in patients with clonal hematopoiesis[6], a strongly age-dependent benign condition in which a single HSC acquires a competitive advantage and produces a large proportion of mature blood cells in the circulation. Thus, Wendorff et al. set out to investigate the role of PHF6 in HSC aging.

Using single-cell RNA sequencing, the authors transcriptionally compared chronologically aged wild-type and *Phf6*-knockout HSCs. Interestingly, the transcriptional landscape of *Phf6*-knockout HSCs is notably different from wild-type HSCs. Aged *Phf6*-knockout HSCs were more similar to young wild-type HSCs, suggesting – at the RNA level – a shift toward a younger transcriptome. Furthermore, the authors examined phenotypical changes of young and aged wild-type and *Phf6*-knockout HSCs. Flow cytometry data demonstrated a partial restoration of various hematopoietic populations in aged *Phf6*-knockout mice, and the absence of the well-known age-associated HSC expansion. *Phf6*-knockout HSCs also demonstrated lower expression of age-associated cell surface markers. Altogether, transcriptomic and phenotypical characterization suggests that aged HSCs lacking PHF6 resemble young rather than aged HSCs.

The popular proverb 'where there is smoke, there must be fire' does not necessarily hold in molecular biology. To prove that aged *Phf6*-knockout HSCs truly are rejuvenated, HSCs must be functionally assessed. The gold standard assay in the field is a HSC transplantation, which is an experiment in which both self-renewal and multilineage differentiation – the very aspects that define stem cells – can be assessed

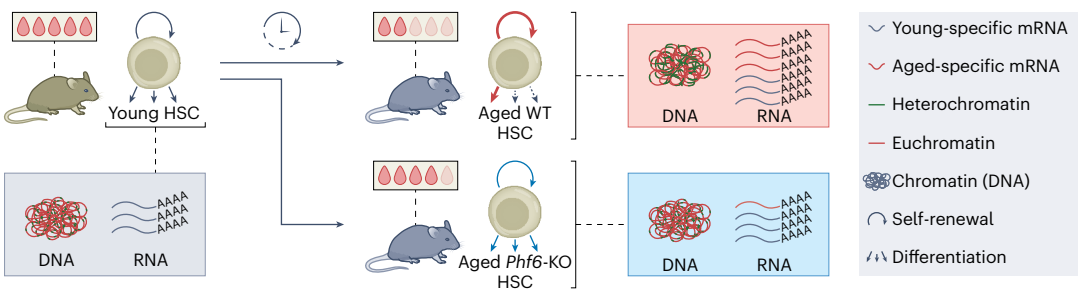

Fig. 1 | *Phf6* inactivation leads to HSC rejuvenation. Left, Young mice have highly functional HSCs with balanced self-renewal and multilineage differentiation. Top right, Upon chronological aging, HSCs become myeloid-biased and their population expands, leading to functional decline. Molecularly, aged HSCs present with accessible chromatin and increased transcription of age-related genes (red box). Bottom right, Upon *Phf6* inactivation, HSC functionality is restored and both chromatin and transcriptional landscape resemble young-like HSCs (blue box). KO, knockout; WT, wild type.

at the same time. To this end, the authors performed transplantations with *Phf6*-depleted HSCs to functionally assess whether these cells would now also behave similarly to young HSCs. Aged *Phf6*-knockout HSCs demonstrated robust functionality, with improved hematopoietic replenishment and diminished myeloid skewing, corroborating that *Phf6* inactivation can ameliorate the decrease of functionality of aged HSCs.

The authors next attempted to rejuvenate age-associated HSC characteristics. To this end, *Phf6* was not depleted at the embryonic stage as in the experiments above, but only after mice had aged for 15 months. Similar to *Phf6*-knockout HSCs, *Phf6* depletion late in life demonstrated a reversal of myeloid skewing and increased repopulation potential. Remarkably, transcriptomic characterization demonstrated that *Phf6*-depleted HSCs molecularly resembled young wild-type HSCs, thus demonstrating that loss of PHF6 can functionally and transcriptionally rejuvenate HSCs. Taken together, this study provides a promising perspective that age-associated functional decline in the hematopoietic system can indeed be restored. At the same time, it also raises interesting future perspectives.

Deregulated gene expression has been assessed by many studies to search for age-related HSC genes. Interestingly, *Phf6* has not been reported to be transcriptionally deregulated upon HSC aging[7]. Therefore, although transcription is an important cellular process, post-transcriptional regulation processes are as relevant and should not be overlooked. Furthermore, *Phf6* has been implicated in the DNA damage response by previous publications and by the current study[3,8]. Nevertheless, the role of PHF6 and how it controls age-dependent mechanisms remains unknown and it is not resolved by the current study. As *Phf6* itself is not deregulated upon aging, how can it account for HSC functional decline? Does it affect alternative splicing, altered protein folding and different age-related protein–protein interactions? In addition, it would be relevant to understand *Phf6* activation in the context of inflammation and bone marrow niche remodeling, as these appear to be important drivers of age-dependent hematopoietic decline[9–11].

It is surprising that inactivation of a single gene is able to functionally restore HSC function. It has been firmly established that aging is a multifactorial process in which several molecular processes are involved. In line with this hypothesis, we recently demonstrated that from a transcriptional perspective there is not a single gene whose expression can function as an reliable aging predictor[7]. Nevertheless, it seems that *Phf6* has a central role in HSC demise and it is a tantalizing possibility that it may function upstream of all these processes. PHF6 is an epigenetic regulator, and thus has the potential to globally regulate HSC fate and therefore its decline. In line with recent results by Itowaka and colleagues[12] and our own work[7], this study confirms increased global chromatin permissiveness in aged HSCs. More importantly, it shows that upon rejuvenation after *Phf6* inactivation, chromatin becomes more compact, suggesting that epigenetic modifications have a causal role in HSC aging and are not merely consequential (Fig. 1).

This study shows that *Phf6* is a crucial factor in HSC aging and calls for further studies aimed at understanding how the different layers of epigenetic modifications (including chromatin remodeling, histone modifications and DNA methylation) are perturbed during HSC aging[13]. Epigenetic modifications are becoming targets for pharmacological intervention, as most of these are reversible and can be modulated by an increasing number of small molecules. Although not assessed by Wendorff et al., further studies should investigate whether *Phf6* has a conserved function in human HSCs and, if so, whether pharmacological inhibition of PHF6 can be used to rejuvenate aged HSCs and thereby improve healthy aging.

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Competing interests

The authors declare no competing interests.