Published in final edited form as:

Semin Hematol. 2017 January ; 54(1): 51–55. doi:10.1053/j.seminhematol.2016.10.005.

Rejuvenation of aged HSCs

Novella Guidi² and Hartmut Geiger^{1,2,3}

¹Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA

²Institute for Molecular Medicine, Ulm University, Ulm, Germany

³aging research center, Ulm University, Ulm, Germany

Abstract

Until recently, there was broad consensus in the stem cell aging field that the phenotype of aged HSCs is fixed—dominated by cell-intrinsic regulatory mechanisms that cannot be altered by pharmacological or genetic means. The conventional thinking was that HSC aging could not be reverted by therapeutic intervention. This paradigm has started to shift dramatically, in large part because hallmarks of aged HSCs have been successfully reverted by distinct experimental approaches by multiple laboratories. We will discuss in this review these hallmarks of HSCs aging and the novel approaches that successfully ameliorated or even reverted aging-associated hallmarks of aged HSCs.

Introduction

Stem cells were initially thought to be endowed with unlimited self-renewal (“rejuvenation”) capacity, and thus exempt from aging. This age-associated decline in stem cell function leads to a decline in the regenerative capacity of humans and mice (1–6), which may limit lifespan (2,7,8). Aging of HSCs is associated with a canonical set of changes in HSCs and their offspring, which will be further introduced and discussed below. These hallmarks of HSCs aging have been reproduced in multiple laboratories. Several reports support that many of these hallmarks/changes are intrinsic to HSCs (9,10), but novel data also support that aging of the BM niche might be able to confer aging-associated phenotypes on HSCs (11–13).

Recent exciting novel publications indicate that distinct undesirable phenotypes associated with aged HSCs can be ameliorated both pharmacologically and genetically (14–20), strongly supporting that the functional decline of aged stem cells may be reversible and that rational interventions can be developed that will target mechanisms of HSC aging to achieve HSC rejuvenation. Identifying conditions under which aged HSCs are activated to become

Correspondence: Novella Guidi and Hartmut Geiger, Institute for Molecular Medicine, University of Ulm, James Franck-Ring 11c, 89081 Ulm, Germany, Phone: +49 731 50 26700 Fax: +49 731 50 26710, novella.guidi@uni-ulm.de or hartmut.geiger@cchmc.org.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

phenotypically and functionally equivalent to young stem cells could be a first step towards designing treatments for age-associated imbalances in hematopoiesis and aging-associated changes in the immune system, thereby allowing for a more healthy aging (21).

1. Hallmarks of aged HSCs

Aging within the hematopoietic system comprises a set of distinct changes, including an increase in the frequency of myeloid cells generated linked to an increase in myeloid-related leukemia, a decrease in the output of lymphoid cells (primarily B-cells) and a reduced regenerative capacity of hematopoiesis upon stress and a decrease in the function of both the innate and adaptive branches of the immune response (9,22,23). One underlying cellular cause of aging of the hematopoietic system is aging of hematopoietic stem cells (HSCs). While upon aging the number of phenotypic HSCs increases, their regenerative potential (as determined in serial transplantation assays) decreases (24). Aged HSCs differentiate preferentially into myeloid cells and show diminished support of the B-cell lineage. Loss of a polar distribution of polarity proteins like Cdc42 and Scribble but also tubulin in the cytoplasm, and loss of the polar distribution of the acetylated form of the epigenetic marker H4K16 in the nucleus, which are caused by an elevated activity of the small RhoGTPase Cdc42 in aged HSCs, are additional recently identified hallmarks of aged HSCs (18,25). Aged HSCs also present with changes in their epigenome and altered gene expression profiles (9,18,22,25). Indeed, genome-wide expression studies comparing HSCs from young and old mice indicated that in aged HSCs, expression of genes involved in myeloid development was increased whereas expression of gene involved in lymphopoiesis was decreased; moreover also gene sets associated with inflammation, stress response and senescence were increased in aged HSC like Clusterin, Platelet-selectin, Cyclooxygenase 2, Toll-like receptor 4 and the Heat shock proteins 5 and 8. (9,10).

Changes in clonality in hematopoiesis upon aging, characterized by an accumulation of a predominantly myeloid-dominant HSC population, demonstrated that individual old HSCs are functionally inferior to their young counterparts in multiple phenotypes, including a lower functional frequency in vitro and in vivo, a delayed proliferation response in stromal co-cultures, a reduced efficiency for short-term BM homing, and a reduced long-term in vivo self-renewal activity (24).

2. Is Impaired genomic integrity and DNA damage linked to aging of HSCs?

DNA repair maintains genomic stability and the loss of DNA repair ability results in genetic instability that may lead to a decline of cellular function. DNA lesions, initiate the DNA damage response (DDR) which comprise mechanism of DNA repair but also signal transduction to induce apoptosis or senescence. DNA lesions thus activate a series of signaling pathways including DNA repair, cell cycle checkpoint activation, senescence, and cell death (26–28). DNA lesions and the DDR also induce chromatin remodeling. While these epigenome and transcriptome modifications brought upon DNA lesions definitely shape the dynamics of the HSCs in the context of self-renewal and differentiation (28–30), the question on whether DNA damage and accumulation of DNA damage with aging contributes to aging of HSCs is still controversially discussed. A number of human genetic

abnormalities associated with aging like Xeroderma Pigmentosa (XP), Cockayne Syndrome (CS) and Trichothiodystrophy (TTD) and those replicated in the mouse like the TTD mouse model and *Ercc1* $-/-$ mice, suggest that loss of DNA repair pathways may contribute to the aging process (31–33). Although there is an about two-to threefold increase in mutation frequency in hematopoiesis upon aging (34,35), the increase might still be seen as minor and presents itself with a linear increase in the frequency over time, which does not correlate with the exponential increase in for example aging-associated leukemia. Modeling approaches of HSC aging, based on evolutionary theories, further suggest that accumulation of genetic changes within HSCs is not sufficient to alter HSC fitness, and instead proposes extrinsic microenvironmental mechanisms as the major selective driving force for aging-associated leukemia (36,37). Additionally, studies conducted in mice deficient for genes involved in several distinct genome maintenance pathways in hematopoiesis like the nucleotide excision repair genes *Xpd* and *Ercc1* or genes involved in telomere maintenance and non-homologous end-joining like *Rad50*^{k22m} and *Ku80* / *Ku70*, have shown loss of reconstitution capacity, diminished self-renewal, increased apoptosis along side with an early exhaustion of the mutant HSCs (32,38,39). As a general decrease in the number of HSCs does not resemble the observation that physiological aging results in an increase in the number of HSC number, the question arises whether aged HSC indeed present with impaired DDR repair pathways. A recent study from our laboratory has demonstrated that the DDR efficiency is not altered upon aging in HSCs in vivo, demonstrated by a similar response to DNA damage of both young and old HSPCs with respect to cell-cycle checkpoints activation and apoptosis and the accumulation of DNA mutations in response to total body irradiation. Moreover, in a competitive transplantation setting, young and old HSPCs showed identical functional activity in response to irradiation. (34). Thus additional experiments are required to determine whether DDR signaling pathways could represent novel targets for therapies aiming to improve tissue integrity and to prevent cancer development during aging.

3. Novel hallmarks of HSC aging: Altered epigenetic architecture and loss of protein polarity

Regulation of transcription is, among others, regulated by epigenetic mechanisms. This is also central for HSC aging in which several genes for myeloid cell differentiation become upregulated on the expense of lymphopoiesis genes which are downregulated (10) and these alterations are maintained also when old HSCs are transplanted into new recipients (40). Importantly, epigenetic changes can be inherited to daughter cells, which is an underlying prerequisite for the accumulation of epigenetic changes in HSCs upon aging, similar to DNA mutations. Several components involved in chromatin reorganization and epigenetic maintenance have been reported to become altered upon aging (9). DNA methylation patterns of old HSCs for instance have been found to be distinct compared to young HSCs, with hypermethylation on regulatory regions associated with HSC differentiation and hypomethylation on genes involved in HSC maintenance (41). Moreover other studies have found that in old HSCs many hypermethylated promoter regions are polycomb group (PcG) target genes and genes regulated by H3K27Me3 and H3K4Me3 histone marks (22,42), which function both to promote and repress gene transcription, respectively. All these epigenetic shifts and chromatin remodeling can be inherited to daughter cells and since most

HSCs undergo cell divisions upon differentiation, this contribute to functional decline of hematopoiesis upon aging.

In a recent study Wang et al, found that PER2 (period circadian rhythm 2), a transcription factor that binds E-boxes is upregulated in HSCs upon aging and contribute to the induction of DDRs and apoptosis and plays a role in lymphoid lineage potential. Moreover, deletion of Per2 in vivo rescued the diminished lymphoid potential and immune function of old HSCs in a cell-intrinsic manner and led to elongated lifespan (20). One explanation for such an observation might be that the transcriptional repression of PER2 on lymphopoietic genes is one of the driving forces for the aging-associated lineage skewing, reinforcing the hypothesis that aged-related changes in HSC are driven and maintained by an altered epigenetic architecture.

Another study from our laboratory demonstrated that also an altered spatial distribution of epigenetic marks in the nucleus contributes to HSC aging. Specifically the distribution of H4K16Ac, which is associated with active gene expression, was found to be apolar in nuclei of old HSCs compared to young HSCs, in which it is mostly polar. Polarity in HSCs in controlled by the activity of the small RhoGTPase Cdc42 which is elevated upon aging; treatment with a specific pharmacological inhibitor of Cdc42 activity (CASIN) restored H4K16Ac polar distribution in old HSCs and re-established a youthful function of aged HSCs (18).

Are these above mentioned changes in gene expression levels that are controlled by changes in epigenetics and/or the spatial distribution of epigenetic marks a driving force or rather a consequence of HSC aging? These are unanswered questions. Their resolution though will directly contribute to novel approaches to ameliorate HSC aging. Taking these data altogether, HSC aging is strongly correlated with changes in epigenetic, transcriptional and polarity landscape.

Aging in hematopoiesis is also influenced, as more recently discovered, by extrinsic factors like changes of the bone marrow niche upon aging. For instance, skewing of aged HSCs towards myeloid differentiation was recently linked to increased secretion of the pro-inflammatory CC-chemokine ligand 5 (CCL5; also known as RANTES) in aged stroma (12). Furthermore SDF-1, an important chemokine produced by osteoblastic cells in the BM niche which is involved in homing, engraftment, neovascularization and cell proliferation, was found to be decreased in MSCs of aged mice (11), suggesting that altered levels of niche-secreted factors may infer HSC aging-associated phenotypes. Also differences in the cellular composition of the aged and young BM niche imply that aging of the niche influences the hematopoietic system (13). Moreover the reduction in HSC adhesion to BM stroma cells upon aging (43) might account for the decrease in HSC self-renewal ability (44). Also the increase in adipocytes upon aging negatively regulates hematopoiesis as it delays engraftment (45). Such extra-medullary adipose tissue cells have been recently reported to contribute to the development of aging-associated leukemia by supporting the metabolic demands of leukemic stem cells (46). So far though changes in the HSC niche upon aging that account for changes in HSCs upon aging are poorly understood (47,48). Is the aging-associated epigenetic drift a stem cell-intrinsic mechanism or is it also affected by

extrinsic micro-environmental changes in the niche? Whether signals from aged niches directly affect the epigenetic landscape and HSC cell polarity is novel field of research. A deeper understanding of mechanisms of niche aging might though shed new light on the distinct mechanisms that contribute extrinsically to aging of HSCs and which might, besides the intrinsic pathways of aging, also become promising targets to ameliorate aging of HSCs.

4. HSC rejuvenation: HSC intrinsic aging mechanisms as a target

Mechanisms of HSC aging involve distinct cell-intrinsic and cell-extrinsic regulatory pathways. The elucidation of all of these likely mechanisms that affect HSC aging will strongly support approaches to rationally target HSC aging in vitro and vivo. The concept of successful rejuvenation approaches was supported by Shinya Yamanaka's lab, which in 2006 established the induced pluripotent stem cell (iPSCs) technology in which the introduction of four specific genes encoding transcription factors could convert adult somatic cells into pluripotent stem cells (49). This discovery suggested that by targeting the epigenetic landscape differentiation can be reverted, which implies that also aging, if primarily driven by epigenetic mechanism, might be reversible. Wahlestedt et al, by reprogramming old HSC into iPSCs and then re-differentiating them into HSCs, demonstrated that the resulting HSCs were functionally similar to young HSCs, thus actually experimentally demonstrating that HSC aging is reversible by epigenetic reprogramming (40).

Here in this section, we summarize and interpret recently published data in which intrinsic mechanisms of aging were targeted both genetically or pharmacologically to achieve at least in part a rejuvenation of the function of aged HSCs (table 1).

Special AT-rich sequence-Binding protein-1 (Satb1)

the chromatin organizer Satb1, an epigenetic regulator of lymphoid progenitor cells, was found to be expressed at a lower level in old HSCs compared to young HSCs. This reduced expression correlated with a decline in HSC function, as overexpression of Satb1 in old HSPCs improved their ability to give rise to a lymphoid progeny in vitro (19). Satb1 might thus be of clinical importance to overcome immunosenescence of older adults by enhancing lymphopoiesis.

Period circadian clock 2 (Per2)

As discussed above, Per2 is upregulated upon aging in lymphoid-biased HSCs and stimulates the DDR and p53-dependent apoptosis. The deletion of Per2 in old HSCs attenuated the aging-associated myeloid/lymphoid skewing of hematopoiesis upon physiological aging as well as telomere shortening in lymphoid cells in vivo. This resulted in an increased immune function and elongated lifespan of prematurely aging mice (20). Per2 might thus be a novel therapeutic target to restore lymphoid potential in an aged HSC compartment.

Sirtuin 3 (Sirt3)

Sirt3, a mammalian sirtuin that regulates mitochondrial de-acetylation, becomes downregulated in HSPCs upon aging, which correlated with an increase in ROS production

and increased in oxidative stress in HSPCs. Interestingly, overexpression of Sirt3 in old HSCs decreased ROS production and increased the reconstitution potential of most blood cell lineages (14), while the relative contribution of myeloid cells remained though at the level of aged HSCs.

Sirtuin 7 (Sirt7)

Sirt7, a mammalian sirtuin that regulates promotor acetylation (histone deacetylase), interacts with nuclear respiratory factor 1 (NRF1), a master regulator of mitochondria. SIRT7 represses NRF1 activity to suppress mitochondrial activity and proliferation. Genetic SIRT7 inactivation caused reduced HSCs quiescence and an increased mitochondrial protein folding stress (PFS(mt)) response and a compromised regenerative capacity of hematopoietic stem cells (HSCs). SIRT7 overexpression or NRF1 inactivation in aged HSCs reduced PFSmt, improved reconstitution capacity, and rescued the myeloid-biased differentiation of aged HSCs. These findings define the deregulation of the mitochondrial stress-mediated metabolic checkpoint as a reversible contributing factor to HSC aging (50).

Mammalian target of Rapamycin (mTOR)

mTOR is an important nutrient-sensing protein which has been implicated on organism longevity (51). mTOR has been reported to have higher activity in old HSCs, which was linked to a relative decrease in lymphopoiesis and an impaired capacity to reconstitute the hematopoietic system (16). Treatment of old mice with the mTOR inhibitor Rapamycin increased their life span, reduced the number HSC to a youthful level, restored youthful HSC self-renewal properties, and resulted in an improved vaccination response against a lethal challenge with influenza virus (16). Because inhibition of mTOR signaling via rapamycin has been also involved in regulating longevity of organisms (52), targeting the mTOR pathway might result in the rejuvenation of not only the hematopoietic system but might also contribute to attenuate aging of whole organism.

Prolonged fasting

Prolonged fasting of aged mice of up to 72 hours has been found to reduce the frequency of myeloid-biased HSCs, promote a youthful HSC self-renewal ability and regenerative capacity upon serial transplantation. Mechanistically, prolonged fasting resulted in reduced circulating IGF-1 levels and reduced PKA activity that correlated with a restoration of youthful levels of HSC intrinsic nutrient sensing (17). Investigations of whether prolonged fasting can improve aging-associated immunosenescence in humans are then of high interest, not only for vaccination approaches but also in the context of tissue regeneration modified by immune cells as well as novel cancer immune-therapy approaches.

Inhibition of Cdc42 activity (by CASIN)

Ex vivo treatment of aged HSCs with CASIN, a specific pharmacological inhibitor of Cdc42 activity, functionally rejuvenated old HSCs with respect to several phenotypes associated with HSC aging (18). Elevated activity in aged HSCs is driven by a shift from canonical to non canonical Wnt signaling due to an increase of Wnt5a expression in old HSCs (53). Experiments are underway to test inhibition of Cdc42 activity in vivo. Elevated Cdc42

activity upon aging is not restricted to the hematopoietic system. Inhibition of Cdc42 activity in vivo might thus also target non-hematopoietic tissues.

ABT263

Chang et al. have recently demonstrated that ABT263, a specific inhibitor of the anti-apoptotic proteins BCL-2 and BCL-xL, induced apoptosis of senescent cell expressing p16 both in culture and in vivo. Oral administration of this potent novel senolytic drug to either sublethally irradiated or aged mice effectively depleted senescent HSCs in the bone marrow. This resulted in the case of old animals in a rejuvenation of the pool of old HSCs, which after treatment with ABT263 showed less myeloid biased and improved reconstitution potential upon serial transplantations (15). Because the ABT263 treatment also abrogated the senescence-associated secretory phenotype (SASP) response in bone marrow stromal cells, it would be of particular interest to investigate whether the rejuvenation effect of ABT263 on old HSCs in vivo might be also due to depletion of senescent cells in the bone marrow microenvironment.

5. Targeting aging of the HSC niche for HSC rejuvenation

Also age-related changes in the local BM niche microenvironment influence the aging-associated decline in HSCs function. Indeed, BM niche signals such as locally secreted cytokines have been already described in regulating HSC fate (54). Age-related changes in stroma secreted factors like CXCL12 (down upon aging) and Rantes/Ccl5 (up upon aging) have been identified to regulate HSC function and myeloid differentiation upon aging (12). Furthermore, an increase of oxidative stress, inflammation and a decrease of the extent of hypoxia have been associated with aging of the niche (55). In general though, the degree by which an aged niche influences HSC aging remains still to be investigated in more detail.

Given a role for the aged niche in conferring aging on HSCs, establishing strategies to target and rejuvenated the aged niche might be a novel tool to rejuvenate HSCs. For example, in a recent study Kathri et al. reported that there is an accumulation of ROS in aged CD45 negative BM stromal cells, which lead to their death via apoptosis (56). Treatment of old stroma cells with curcumin, a natural antioxidant agent, reduced these high ROS levels and subsequently the high levels of apoptosis, and reduced the expression of the stress-responsive genes *Nox-1*, *Sod-2* and *Gpx-1*. Moreover, treatment of 18 month old animals with curcumin improved BM cellularity and thus hematopoietic reconstitution upon transplantation of bone marrow cells compared to transplants into old non-treated animals. Another more recent study demonstrated that sex steroid ablation (SSA) induced hematopoietic and lymphoid recovery by enhancing self-renewal of old HSC as well as lymphoid differentiation through both HSCs intrinsic mechanisms but also via upregulation of factors like *Opn*, *Ang1*, *Cxcl12*, *Scf*, *Tgfb*, *Jag1* and *Vcam1* in endothelial cells, pre-osteoblasts and mature osteoblasts of old mice. Many of these factors are directly regulated by FOXO1, an known aging gene (57). In summary, old mice showed a significant shift in gene expression after SSA from a profile characteristic of an old mouse to that of a young mouse 10 days after SSA. Collectively these data imply that also modulation of factors in the niche can offer novel targeted strategies for HSC rejuvenation.

6. Conclusions/Outlook

Recent developments have identified molecular mechanisms of aging of HSCs, driven both by cell intrinsic mechanisms as well as by aging of the stem cell niche. Based on these findings, genetic or pharmacological approaches were developed. They demonstrate that unwanted aging-associated changes of HSCs function can be ameliorated or sometimes even reverted to a youthful level and thus rejuvenated. These are promising findings towards designing treatments for age-associated imbalances in hematopoiesis and aging-associated changes in the immune system towards a more healthy aging. There is evidence that an aged hematopoietic and immune system might also affect aging-associated changes in solid organs. For example, aging-associated reduced clonality in the human hematopoietic system strongly correlated to a higher incidence of cardiovascular disease (58). Novel experiments are thus warranted to test in as much a rejuvenated hematopoietic system might also be able to contribute to amelioration of unwanted aging-associated changes in other organ systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Work in the laboratory of H. Geiger was supported by National Institutes of Health Grants HL076604, DK077762, AG040118; the Deutsche Forschungsgemeinschaft (DFG; SFB 1074 and 1149, KFO142); the German Federal Ministry of Education and Research (BMBF; SyStaR, grant no. 0315894A); the Excellence program of the Baden-Württemberg Foundation; and funds from the German Scholar organization (GSO) and the European Commission (FP7 Marie Curie Initial Training Network MARRIAGE, grant no. 316964). The authors declare no conflicting financial interests.

References

1. Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature*. 2005 Feb 17; 433(7027): 760–4. [PubMed: 15716955]
2. Geiger H, Van Zant G. The aging of lympho-hematopoietic stem cells. *Nat Immunol*. 2002 Apr; 3(4):329–33. [PubMed: 11919569]
3. Kim M, Moon H-B, Spangrude GJ. Major age-related changes of mouse hematopoietic stem/progenitor cells. *Ann N Y Acad Sci*. 2003 May; 996:195–208. [PubMed: 12799297]
4. Kirkwood TBL. Intrinsic ageing of gut epithelial stem cells. *Mech Ageing Dev*. 2004 Dec; 125(12): 911–5. [PubMed: 15563938]
5. Potten CS, Martin K, Kirkwood TB. Ageing of murine small intestinal stem cells. *Novartis Found Symp*. 2001; 235:66–79. 79–84, 101–4. [PubMed: 11280034]
6. Sudo K, Ema H, Morita Y, Nakauchi H. Age-associated characteristics of murine hematopoietic stem cells. *J Exp Med*. 2000 Nov 6; 192(9):1273–80. [PubMed: 11067876]
7. Sharpless NE, DePinho RA. Telomeres, stem cells, senescence, and cancer. *J Clin Invest*. 2004 Jan; 113(2):160–8. [PubMed: 14722605]
8. Torella D, Rota M, Nurzynska D, Musso E, Monsen A, Shiraishi I, et al. Cardiac stem cell and myocyte aging, heart failure, and insulin-like growth factor-1 overexpression. *Circ Res*. 2004 Mar 5; 94(4):514–24. [PubMed: 14726476]
9. Chambers SM, Shaw CA, Gatz C, Fisk CJ, Donehower LA, Goodell MA. Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation. *PLoS Biol*. 2007 Aug; 5(8):e201. [PubMed: 17676974]

10. Rossi DJ, Bryder D, Zahn JM, Ahlenius H, Sonu R, Wagers AJ, et al. Cell intrinsic alterations underlie hematopoietic stem cell aging. *Proc Natl Acad Sci U S A*. 2005 Jun 28; 102(26):9194–9. [PubMed: 15967997]
11. Choudhery MS, Khan M, Mahmood R, Mehmood A, Khan SN, Riazuddin S. Bone marrow derived mesenchymal stem cells from aged mice have reduced wound healing, angiogenesis, proliferation and anti-apoptosis capabilities. *Cell Biol Int*. 2012 Aug 1; 36(8):747–53. [PubMed: 22352320]
12. Ergen, AV., Boles, NC., Goodell, MA. Rantes/Ccl5 influences hematopoietic stem cell subtypes and causes myeloid skewing. *Blood* [Internet]. 2012 Jan 30. [cited 2012 Feb 6]; Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22289892>
13. Wagner W, Horn P, Bork S, Ho AD. Aging of hematopoietic stem cells is regulated by the stem cell niche. *Exp Gerontol*. 2008 Nov; 43(11):974–80. [PubMed: 18504082]
14. Brown K, Xie S, Qiu X, Mohrin M, Shin J, Liu Y, et al. SIRT3 reverses aging-associated degeneration. *Cell Rep*. 2013 Feb 21; 3(2):319–27. [PubMed: 23375372]
15. Chang J, Wang Y, Shao L, Laberge R-M, Demaria M, Campisi J, et al. Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. *Nat Med*. 2016 Jan; 22(1):78–83. [PubMed: 26657143]
16. Chen C, Liu Y, Liu Y, Zheng P. mTOR regulation and therapeutic rejuvenation of aging hematopoietic stem cells. *Sci Signal*. 2009; 2(98):ra75. [PubMed: 19934433]
17. Cheng C-W, Adams GB, Perin L, Wei M, Zhou X, Lam BS, et al. Prolonged fasting reduces IGF-1/PKA to promote hematopoietic-stem-cell-based regeneration and reverse immunosuppression. *Cell Stem Cell*. 2014 Jun 5; 14(6):810–23. [PubMed: 24905167]
18. Florian MC, Dörr K, Niebel A, Daria D, Schrezenmeier H, Rojewski M, et al. Cdc42 activity regulates hematopoietic stem cell aging and rejuvenation. *Cell Stem Cell*. 2012 May 4; 10(5):520–30. [PubMed: 22560076]
19. Satoh Y, Yokota T, Sudo T, Kondo M, Lai A, Kincade PW, et al. The Satb1 protein directs hematopoietic stem cell differentiation toward lymphoid lineages. *Immunity*. 2013 Jun 27; 38(6):1105–15. [PubMed: 23791645]
20. Wang J, Morita Y, Han B, Niemann S, Löffler B, Rudolph KL. Per2 induction limits lymphoid-biased haematopoietic stem cells and lymphopoiesis in the context of DNA damage and ageing. *Nat Cell Biol*. 2016 May; 18(5):480–90. [PubMed: 27088856]
21. Geiger H, Zheng Y. Cdc42 and aging of hematopoietic stem cells. *Curr Opin Hematol*. 2013 Jul; 20(4):295–300. [PubMed: 23615056]
22. Beerman I, Bock C, Garrison BS, Smith ZD, Gu H, Meissner A, et al. Proliferation-dependent alterations of the DNA methylation landscape underlie hematopoietic stem cell aging. *Cell Stem Cell*. 2013 Apr 4; 12(4):413–25. [PubMed: 23415915]
23. Miller JP, Allman D. Linking age-related defects in B lymphopoiesis to the aging of hematopoietic stem cells. *Semin Immunol*. 2005 Oct; 17(5):321–9. [PubMed: 15979895]
24. Dykstra B, Olthof S, Schreuder J, Ritsema M, de Haan G. Clonal analysis reveals multiple functional defects of aged murine hematopoietic stem cells. *J Exp Med*. 2011 Dec 19; 208(13):2691–703. [PubMed: 22110168]
25. Florian MC, Geiger H. Concise review: polarity in stem cells, disease, and aging. *Stem Cells Dayt Ohio*. 2010 Sep; 28(9):1623–9.
26. Kenyon J, Gerson SL. The role of DNA damage repair in aging of adult stem cells. *Nucleic Acids Res*. 2007; 35(22):7557–65. [PubMed: 18160407]
27. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. *Nature*. 2009 Oct 22; 461(7267):1071–8. [PubMed: 19847258]
28. Li T, Zhou Z-W, Ju Z, Wang Z-Q. DNA Damage Response in Hematopoietic Stem Cell Ageing. *Genomics Proteomics Bioinformatics*. 2016 Jun; 14(3):147–54. [PubMed: 27221660]
29. Mandal PK, Blanpain C, Rossi DJ. DNA damage response in adult stem cells: pathways and consequences. *Nat Rev Mol Cell Biol*. 2011 Mar; 12(3):198–202. [PubMed: 21304553]
30. Blanpain C, Mohrin M, Sotiropoulou PA, Passegué E. DNA-damage response in tissue-specific and cancer stem cells. *Cell Stem Cell*. 2011 Jan 7; 8(1):16–29. [PubMed: 21211780]
31. Lehmann AR. DNA repair-deficient diseases, xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy. *Biochimie*. 2003 Nov; 85(11):1101–11. [PubMed: 14726016]

32. Prasher JM, Lalai AS, Heijmans-Antonissen C, Ploemacher RE, Hoeijmakers JHJ, Touw IP, et al. Reduced hematopoietic reserves in DNA interstrand crosslink repair-deficient *Ercc1*^{-/-} mice. *EMBO J*. 2005 Feb 23; 24(4):861–71. [PubMed: 15692571]
33. Vermeulen W, Bergmann E, Auriol J, Rademakers S, Frit P, Appeldoorn E, et al. Sublimiting concentration of TFIIF transcription/DNA repair factor causes TTD-A trichothiodystrophy disorder. *Nat Genet*. 2000 Nov; 26(3):307–13. [PubMed: 11062469]
34. Moehrle BM, Nattamai K, Brown A, Florian MC, Ryan M, Vogel M, et al. Stem Cell-Specific Mechanisms Ensure Genomic Fidelity within HSCs and upon Aging of HSCs. *Cell Rep*. 2015 Dec 22; 13(11):2412–24. [PubMed: 26686632]
35. Vijg J, Busuttil RA, Bahar R, Dollé MET. Aging and genome maintenance. *Ann N Y Acad Sci*. 2005 Dec.1055:35–47. [PubMed: 16387716]
36. Möhrle B, Geiger H. Aging of hematopoietic stem cells: DNA damage and mutations? *Exp Hematol*. 2016 Jul 8.
37. Rozhok AI, Salstrom JL, DeGregori J. Stochastic modeling reveals an evolutionary mechanism underlying elevated rates of childhood leukemia. *Proc Natl Acad Sci U S A*. 2016 Jan 26; 113(4): 1050–5. [PubMed: 26755588]
38. Bender CF, Sikes ML, Sullivan R, Huye LE, Le Beau MM, Roth DB, et al. Cancer predisposition and hematopoietic failure in *Rad50*(S/S) mice. *Genes Dev*. 2002 Sep 1; 16(17):2237–51. [PubMed: 12208847]
39. Rossi DJ, Bryder D, Seita J, Nussenzweig A, Hoeijmakers J, Weissman IL. Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. *Nature*. 2007 Jun 7; 447(7145):725–9. [PubMed: 17554309]
40. Wahlestedt M, Norddahl GL, Sten G, Ugale A, Frisk M-AM, Mattsson R, et al. An epigenetic component of hematopoietic stem cell aging amenable to reprogramming into a young state. *Blood*. 2013 May 23; 121(21):4257–64. [PubMed: 23476050]
41. Sun D, Luo M, Jeong M, Rodriguez B, Xia Z, Hannah R, et al. Epigenomic profiling of young and aged HSCs reveals concerted changes during aging that reinforce self-renewal. *Cell Stem Cell*. 2014 May 1; 14(5):673–88. [PubMed: 24792119]
42. Taiwo O, Wilson GA, Emmett W, Morris T, Bonnet D, Schuster E, et al. DNA methylation analysis of murine hematopoietic side population cells during aging. *Epigenetics*. 2013 Oct; 8(10):1114–22. [PubMed: 23949429]
43. Köhler A, Schmithorst V, Filippi M-D, Ryan MA, Daria D, Gunzer M, et al. Altered cellular dynamics and endosteal location of aged early hematopoietic progenitor cells revealed by time-lapse intravital imaging in long bones. *Blood*. 2009 Jul 9; 114(2):290–8. [PubMed: 19357397]
44. Mendelson A, Frenette PS. Hematopoietic stem cell niche maintenance during homeostasis and regeneration. *Nat Med*. 2014 Aug; 20(8):833–46. [PubMed: 25100529]
45. Naveiras O, Nardi V, Wenzel PL, Hauschka PV, Fahey F, Daley GQ. Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment. *Nature*. 2009 Jul 9; 460(7252):259–63. [PubMed: 19516257]
46. Ye H, Adane B, Khan N, Sullivan T, Minhajuddin M, Gasparetto M, et al. Leukemic Stem Cells Evade Chemotherapy by Metabolic Adaptation to an Adipose Tissue Niche. *Cell Stem Cell*. 2016 Jul 7; 19(1):23–37. [PubMed: 27374788]
47. Ergen AV, Goodell MA. Mechanisms of hematopoietic stem cell aging. *Exp Gerontol*. 2010 Apr; 45(4):286–90. [PubMed: 20034552]
48. Gopinath SD, Rando TA. Stem cell review series: aging of the skeletal muscle stem cell niche. *Aging Cell*. 2008 Aug; 7(4):590–8. [PubMed: 18462272]
49. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006 Aug 25; 126(4):663–76. [PubMed: 16904174]
50. Mohrin M, Shin J, Liu Y, Brown K, Luo H, Xi Y, et al. Stem cell aging. A mitochondrial UPR-mediated metabolic checkpoint regulates hematopoietic stem cell aging. *Science*. 2015 Mar 20; 347(6228):1374–7. [PubMed: 25792330]
51. Johnson SC, Rabinovitch PS, Kaeberlein M. mTOR is a key modulator of ageing and age-related disease. *Nature*. 2013 Jan 17; 493(7432):338–45. [PubMed: 23325216]

52. Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature*. 2009 Jul 16; 460(7253):392–5. [PubMed: 19587680]
53. Florian MC, Nattamai KJ, Dörr K, Marka G, Uberle B, Vas V, et al. A canonical to non-canonical Wnt signalling switch in haematopoietic stem-cell ageing. *Nature*. 2013 Nov 21; 503(7476):392–6. [PubMed: 24141946]
54. Lo Celso C, Scadden DT. The haematopoietic stem cell niche at a glance. *J Cell Sci*. 2011 Nov 1; 124(Pt 21):3529–35. [PubMed: 22083139]
55. Petit I, Szyper-Kravitz M, Nagler A, Lahav M, Peled A, Habler L, et al. G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. *Nat Immunol*. 2002 Jul; 3(7):687–94. [PubMed: 12068293]
56. Khatri R, Krishnan S, Roy S, Chattopadhyay S, Kumar V, Mukhopadhyay A. Reactive Oxygen Species Limit the Ability of Bone Marrow Stromal Cells to Support Hematopoietic Reconstitution in Aging Mice. *Stem Cells Dev*. 2016 Jun 15; 25(12):948–58. [PubMed: 27140293]
57. Khong DM, Dudakov JA, Hammett MV, Jurblum MI, Khong SML, Goldberg GL, et al. Enhanced hematopoietic stem cell function mediates immune regeneration following sex steroid blockade. *Stem Cell Rep*. 2015 Mar 10; 4(3):445–58.
58. Baker DJ, Childs BG, Durik M, Wijers ME, Sieben CJ, Zhong J, et al. Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. *Nature*. 2016 Feb 11; 530(7589):184–9. [PubMed: 26840489]

Table 1

Interventions that contribute to HSCs rejuvenation

Target proteins	Type of intervention	Rejuvenation phenotype(s)	Ref.
Satb1	genetic	Overexpression enhances lymphoid progeny	(19)
Per2	genetic	Deletion attenuates lymphoid/myeloid skewing	(20)
Sirt3	genetic	Overexpression decreases ROS production Increases the reconstitution potential	(14)
Sirt7	genetic	Overexpression reduces PFSmt Improves reconstitution capacity Rescues myeloid-biased differentiation	(50)
mTOR	pharmacologic	Inhibition increases life span Reduces the number HSC to a youthful level Restores youthful HSC self-renewal upon transplantation	(16,52)
Prolonged fasting	pharmacologic	Reduces the frequency of myeloid-biased HSCs Restores youthful HSC self-renewal ability and regenerative capacity upon serial transplantation	(17)
Cdc42	pharmacologic	Inhibition enhances competitive repopulation Reduces the frequency of myeloid-biased HSCs	(18)
BCL-2			
BCL-xL	pharmacologic	Inhibition depletes senescent HSCs in the BM Attenuates lymphoid/myeloid skewing Improves reconstitution potential of HSCs upon serial transplantation	(15)