Bone 119 (2019) 1–7

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Review Article

Impact of aging on bone, marrow and their interactions

Corey M. Hoffman, Jimin Han, Laura M. Calvi*

University of Rochester Medical Center, Rochester, NY, United States of America

ARTICLE INFO

Keywords:
Hematopoiesis
Stem cells
Aging
Bone marrow
Microenvironment

ABSTRACT

Hematopoiesis in land dwelling vertebrates and marine mammals occurs within the bone marrow, continually providing mature progeny over the course of an organism's lifetime. This conserved dependency highlights the critical relationship between these two organs, yet the skeletal and hematopoietic systems are often thought of as separate. In fact, data are beginning to show that skeletal disease pathogenesis influences hematopoiesis and viceversa, offering novel opportunities to approach disease affecting bone and blood. With a growing global population of aged individuals, interest has focused on cell autonomous changes in hematopoietic and skeletal systems that result in dysfunction. The purpose of this review is to summarize the literature on aging effects in both fields, and provide critical examples of organ cross-talk in the aging process.

1. Introduction

Hematopoiesis in land-dwelling vertebrates and marine mammals occurs within the bone marrow, continually providing mature progeny over the course of an organism's lifetime. This conserved evolutionary compartmentalization highlights the critical relationship between these two organs. While one cannot exist without the other in humans, bone and marrow are often thought of as separate systems, with each having its own distinct field of research. And yet, these organs interface anatomically. Moreover, data are emerging showing that disease pathogenesis in the skeleton influences the hematopoietic system and viceversa. Based on these findings, the reciprocal interactions of the skeleton and the hematopoietic system are beginning to be appreciated, and were recently reviewed in detail elsewhere [1,2]. With a growing global population of aged individuals, interest has focused on cell autonomous changes in hematopoietic and skeletal systems. However, the emergence of a regulatory role in marrow regulatory microenvironmental cell populations in the bone marrow suggests that dysfunction of the aged bone marrow microenvironment may contribute to aging changes displayed by hematopoiesis. The purpose of this review is to summarize the literature on aging effects in both fields, and provide critical examples of organ cross-talk in the aging process.

2. Cell autonomous changes in hematopoiesis

Aging is a universal process that affects many organs with similar outcomes, including loss of function and greater risk of disease pathogenesis [3]. In the hematopoietic system, aging is associated with increased anemias [4,5], impaired adaptive immune responses [6], and increased susceptibility to myelodysplastic and myeloproliferative disorders [7]. To date, most research has focused on the changes that occur within hematopoietic stem cells (HSCs) over time, leading to cell-autonomous decreased function [8,9]. Well-described features of aged HSCs include changes in surface marker expression [10,11], myeloid and megakaryocytic bias (loss of lymphoid support) [11,12], accumulation of DNA damage [13–15], expansion of the HSC pool [9] and increase of dominant clones [16–19].

2.1. Myeloid and megakaryocytic skewing of hematopoietic stem cells with aging

Myeloid differentiation (neutrophil, monocyte, macrophage, *etc*.) is preferred over lymphoid differentiation (B-cell, T-Cell, natural killer, *etc*.) in the aged hematopoietic system. This is a poorly understood phenomenon referred to as myeloid-bias. HSC phenotyping with various markers has been used to investigate if the aged phenotype correlates with diminished function, distinct clones, or myeloid-bias. For example, the marker CD41/integrin alpha-2B (ITGA2B) is typically associated with platelets and megakaryocytes [10]. Its expression expands into the HSC pool with age and is associated with cells that have a myeloid bias [10,20,21]. Another marker also associated with the megakaryocytic arm of the myeloid lineage shown to expand in aged HSCs is von Willebrand Factor (VWF). VWF expression on HSCs is also functionally associated with megakaryocytic bias in the form of increased platelet output. Myeloid bias and expansion of CD41 + HSCs is associated with increased rounds of self-renewal and aging [22] but

* Corresponding author at: University of Rochester Medical Center, 601 Elmwood Avenue, Box 693, Rochester NY 14642, United States of America.
E-mail address: laura_calvi@urmc.rochester.edu (L.M. Calvi).

https://doi.org/10.1016/j.bone.2018.07.012
Received 20 April 2018; Received in revised form 12 July 2018; Accepted 12 July 2018
Available online 17 July 2018

whether the increase in CD41 + HSCs is increased expression of CD41 in previously CD41- HSCs induced by aging, expression of the HSC phenotype by CD41 + myeloid progenitors or expansion of the CD41 + HSC subset remains controversial. Analysis of human marrow has also yielded evidence for myeloid and megakaryocytic skewing with age [12]. Human marrow has fewer lymphoid progenitors with age, and aged human HSCs have myeloid primed transcriptional programs [12]. Further work is necessary to refine the definition of myeloid skewing, as this could occur with the loss of lymphoid support or changes in lymphopoiesis without any alteration to the myeloid lineage.

2.2. DNA repair and HSC aging

DNA damage is another highly investigated topic in HSC aging. Inherited bone marrow failure syndromes, such as Fanconi Anemia, underscore the importance of genomic stability in hematopoiesis [23]. Mouse models lacking components of different DNA repair pathways, including non-homologous end joining, maintenance of telomeres, and nucleotide excision repair, have reduced long term HSC (LT-HSC) function, and lack the ability to engraft into lethally irradiated recipients [13,15]. Lack of DNA repair processes in these models is also associated with higher levels of apoptosis in the stem cell compartment [13]. Analysis of DNA damage in human HSCs *ex vivo* has also been performed to measure the amount of marker γH2AX (a chromatin mark associated with areas of DNA damage) [14]. HSCs from individuals 50 years and older had higher levels of γH2AX compared to those less than 50 years of age [14]. γH2AX markers were not cleared as quickly in aged HSCs compared to young HSCs when exposed to ionizing radiation [14], underscoring age-dependent cell-intrinsic defects in DNA repair in HSCs. Recent evidence in mice would suggest that γH2AX marks at baseline in aged HSCs do not overlap with markers DNA damage [24], and therefore may represents a separate pathology, which is associated with replication stress.

2.3. HSC pool expansion

Data have shown that phenotypic HSCs expand with age in the human marrow [12]. This phenotype is conserved in C57BL/6 mice [9,25,26], although this finding is strain specific [27,28]. Notably, in spite of this increase in HSC numbers, individual HSCs demonstrate age-dependent decreased function in their ability to engraft and reconstitute hematopoiesis [26,29–31]. Aged human HSCs also share this reduced capacity to engraft [32], suggesting that aged human HSCs have decreased ability to self-renew. The mechanisms underlying both the phenotypic HSC expansion and the HSC decrease in self renewal reported with age remain poorly understood.

2.4. Clonal hematopoiesis

The lack of diversity in the cells that provide progeny to the peripheral blood is referred to as clonal hematopoiesis [18]. Clonal hematopoiesis has been verified in humans, and may be associated with risk of hematopoietic malignancy [17,19]. Identification of clonal populations in the blood is assessed by sequencing over-represented somatic mutations. Further work has focused on distinct mutations that are overrepresented in clonal hematopoiesis [19]. Multiple studies using sequencing found that DNMT3A-R882 and *JAK2*-V617F mutations, which are associated with leukemia, are over represented in clonal populations [19,33,34]. Technological advances have allowed for sequencing of circulating hematopoietic progenitors from a large number of individuals, and now demonstrate that mutations typically associated with hematologic malignancies are relatively common in elderly populations that do not necessarily display evidence of hematologic disorders [18,33,34]. Analysis of the bone marrow from a 115 year old woman demonstrated an extreme example of clonal hematopoiesis in the absence of hematopoietic malignancy. Holstege et al. sequenced the somatic mutations unique to the hematopoietic system in this individual and found that the majority (~65%) of the aged marrow was derived from two related HSC populations. The authors speculate that one HSC population is derived from the other, as they share a subset of somatic mutations [16]. The term Clonal Hematopoiesis of Indeterminate Potential (CHIP) is now used in this setting to highlight the fact that the presence of these mutations is often not associated with hematologic disease in elderly patients, [35]. CHIP is relatively common in aged individuals, as between 10- and 20% of patients over 70 years of age have been reported to have CHIP [18,33,34]. Notably, CHIP is correlated with increased cardiovascular risk [18]. Recent studies in a murine model where introduction of the Tet2 mutation accelerates atherosclerosis provide initial support for a causal role of CHIP in cardiovascular disease. However, more work will be necessary to assess the potentially necessary and/or sufficient components of clonal hematopoiesis in disease pathogenesis. From the point of view of bone-marrow interactions, it is important to consider whether changes in skeletal populations may be responsible for the selection pressures that result in clonal hematopoiesis.

3. Aging of the skeleton

Age-associated skeletal pathologies, including osteoporosis and elevated fracture risk, represent a public health issue not only because of their significant morbidity and mortality to the elderly population, but also because these pathologies incur large medical costs [36]. A greater understanding of the underlying pathology that leads to these changes may therefore indicate potential new therapeutic strategies to mitigate the impact of aging on skeletal health.

3.1. Impact of aging on bone matrix

Osteoporosis is associated with a functional decline in bone formation while maintaining the same level or increasing levels of bone resorption [37]. More recent evidence suggests that osteoporosis results both from a loss in bone matrix and changes in matrix quality [38–41]. Matrix quality can be broadly defined as the components that contribute to the bone matrix [38]. Bone mineral density, the standard for assessing risk and diagnosing osteoporosis, is measured clinically primarily by Dual-energy X-ray absorptiometry (DXA). However, other spectrographic methods are being used now to determine bone composition and diagnose osteoporosis and fracture risk [38,39,42]. Specifically, Fourier-transform infrared spectroscopy imaging is being used to measure bone composition, which includes mineral/matrix ratio, carbonate/phosphate ratio, crystallinity, and collagen maturity (amount of collagen cross-links) [42]. These parameters can thus be measured in bone biopsies and correlated with bone fracture occurrence. Since bone forming cells are primarily responsible for laying down bone matrix, changes within the osteoblast itself during the aging process can lead to changes in both the amount and quality of bone matrix.

3.2. Impact of aging on bone forming cells

The decline in osteoblast function has been associated with changes in mitochondrial function. MSCs require mitochondrial biogenesis in order to effectively undergo osteoblastic differentiation [43]. Evidence *in vitro* also suggests that, during cell division, newly synthesized mitochondria are preferentially split into the daughter stem-cell, rather than the more mature daughter cell, providing further evidence that a "healthy" population of mitochondria is essential for stemness [44]. Dysfunction in the mitochondria is often the result of opening of the mitochondrial permeability transition pore, which is regulated by the protein cyclophilin-D (cypD) [45]. When mice with a global deletion for cypD (cypD-KO) are aged (13 months), they do not exhibit bone loss or osteoporosis compared to wild-type (WT) animals [46]. Bones from

these aged cypD-KO mice also maintain physical properties of young bone, with no change in bone toughness or strength relative to 3 month old cypD-KO [46]. Thus, cypD represents a potential future target for osteoporosis therapies, as it can be inhibited by cyclosporin-A [47,48], which is already shown to be safe for use in humans [49]. Cyclosporin-A has also been shown to protect stem-cell populations from oxidative stress, which negatively impacts the long-term repopulation capacity associated with stemness [50].

In aged bone marrow there is also an accumulation of marrow fat, which shares common progenitors with osteoblasts [51]. Changes in microRNA (miR) expression have been linked to functional changes in osteoblasts that result in osteoporotic phenotypes and favor adipogenesis in mice [52]. Specifically, miR-188 is upregulated in both murine and human osteoblasts during the aging process [52]. Upregulation of miR-188 suppresses the expression of *histone deacetylase 9* (*HDAC9*) [53] and *rapamycin-insensitive companion of mammalian target of rapamycin* (*Rictor*) [54], which are necessary to guide MSCs towards osteoblastic fate and away from adipocytic differentiation [52]. Use of an anti-sense RNA oligonucleotides [55] may represent a potential therapeutic strategy to treat osteoporosis, since Li et al. showed that blocking miR-188 with the anti-sense sequence facilitated bone formation in mice [52].

3.3. Impact of aging on marrow adipocytes

The expansion of adipocytic populations within the marrow may also contribute to bone fragility seen in aging [56]. The authors attribute adipocyte expansion to the increased expression of dipeptidyl peptidase-4 (DPP4) in bone marrow adipocytes (BMA) [56,57]. BMAs expressing DPP4 are expanded with age [56]. Transplantation of BMA precursors into the site of fracture negatively impacted healing, resulting in formation of a cartilaginous callous rather than mineralized tissue [56]. The negative impact of BMA precursors could be abrogated by DPP4 inhibition. However, further work is necessary to see if DPP4 inhibition can also reverse or manage osteoporosis in aged populations.

4. Key signals impacting bone and marrow aging

4.1. Wnt signaling

The canonical Wnt signaling pathway is essential in development, and is dysregulated in disease pathogenesis [58]. In the hematopoietic system, the canonical Wnt pathway has been implicated in the maintenance and regeneration of the HSC compartment [59,60]. Treatment of HSCs with prostaglandin E2 (PGE_2) enhances both HSC self-renewal capacity and function, in part by engagement of the canonical Wnt pathway [60,61]. The positive effects of PGE_2 on HSC expansion could be blocked *in vivo* by inhibiting various components of the canonical Wnt pathway. As part of the Wnt signaling pathway, the adenomatous polyposis coli (APC) complex negatively regulates Wnt signaling. Loss of function APC mutations inversely correlate with different levels of β-catenin activity [62,63]. In studies where different APC mutations were examined, intermediate doses of Wnt with mild APC mutations facilitate HSC differentiation, whereas high doses of Wnt prevent HSC proliferation and self-renewal capacity, suggesting that the degree of Wnt activation may have different impact on the HSC pool [62,63]. The non-canonical Wnt pathway (β-catenin independent) has also been shown to be critical for HSC maintenance [64]. Receptors like Flamingo (*Fmi*) and Frizzled8 (*Fz8*) have been shown to be essential for LT-HSC function *in vivo* [64]. Knock-out mice for either gene have altered cell cycle kinetics with a more proliferative stem cell pool, with a greater proportion existing outside of G_0 in the cell cycle [64]. When HSCs from a murine model of loss of *Fmi* or *Fz8* were transplanted in wildtype recipients, they were deficient in their ability to contribute to the peripheral blood and bone marrow [64]. Mechanistically, *Fmi* and *Fz8* maintain LT-HSC quiescence by suppressing canonical Wnt signaling and nuclear localization of Nuclear Factor of Activated T-cells (NFAT), which would induce expression of Interferon gamma (*IFN*γ) and subsequent HSC activation [64].

While the previous studies highlight the role of both canonical and non-canonical Wnt signaling on HSCs at homeostasis, the literature is just beginning to investigate these pathways in the aging process [65]. Work from the Geiger laboratory suggests there is a balance of canonical and non-canonical Wnt signaling at baseline that is disrupted during aging [65]. Expression of Wnt5a increases in aged LT-HSCs, resulting in autocrine activation of non-canonical Wnt signaling [65]. Wnt5a then engages the Cdc42 pathway, inducing cytoskeletal polarity defects previously shown to negatively impact HSC function [65,66]. By reducing expression of Wnt5a in aged LT-HSCs, the polarity defect and myeloid bias seen in aging were reversed [65]. Overall, these studies highlight that both canonical and non-canonical Wnt signaling are essential for HSC maintenance, and disruption of this balance in aging negatively impacts HSC function.

The Wnt pathway has also been highly studied in bone, where it is also dysregulated by the aging process [67]. The canonical Wnt pathway is essential for bone formation in humans, and is regulated by expression of bone derived inhibitors, Sclerostin [68] (*Sost*) and Dickkopf1 [69] (*Dkk1*), which antagonize the Wnt co-receptors LDL-receptor protein 5 and 6 (*Lrp5/6*). Mutations in Lrp5 have been associated with skeletal phenotypes in humans: loss of function mutations in *LRP5* result in osteoporosis-pseudoglioma syndrome [70], and gain of function mutations result in high bone mass phenotypes that manifest with high BMD and changes in jaw structure [71]. The Lrp5 G171V phenotype exerts its high bone mass phenotype by preventing binding of Dkk1, which antagonizes Wnt signaling [71]. Other high bone mass phenotypes have been associated with mutations in the *SOST* gene which result in sclerosteosis [72] and van Buchem disease [73]. Since loss of function of either *DKK1* or *SOST* results in high bone mass phenotypes, inhibiting these signals may be a strategy to treat osteoporosis in elderly populations, specifically in post-menopausal women [74–78]. In addition to mutations in Wnt receptors, loss of function in Wnt ligands, specifically *Wnt1*, are associated with osteogenesis imperfecta, and result in an early onset of osteoporosis [79]. These mutations induce a dominant negative Wnt1 protein, which is unable to positively modulate β-catenin levels [79]. Thus, human studies have highlighted that loss of canonical Wnt signaling in bone prematurely ages the skeleton and that reengagement of the pathway can reverse these changes. Whether loss of Wnt signals from the skeleton is responsible for the age-dependent loss of HSC function remains unexplored.

4.2. Osteopontin, megakaryocytes and osteomacs

The extracellular matrix protein osteopontin (OPN) is expressed by many tissues. However, in the bone marrow, OPN is primarily expressed by osteoblastic cells [80], where it acts as a component of the bone matrix. OPN is able to regulate both MSC fate choice and HSC pool size [81]. OPN directs MSC fate choice towards osteogenesis and away from adipocytic differentiation through binding the integrin heterodimer $\alpha_V\beta_1$ (CD51/CD29) on MSCs. Use of antibodies against OPN, and against either α_V or β_1 integrins, impaired osteogenic differentiation and enhanced adipogenic differentiation *in vitro* [81]. MSCs from mice lacking expression of OPN (OPN-KO mice) also recapitulated this *in vitro* effect, but the investigators did not analyze these populations *in vivo* in the bone marrow [81].

OPN in the bone marrow also acts to restrain the size of the HSC pool; with OPN-KO animals having ~2 fold increase in HSCs numbers [82]. The HSC pool in OPN-KO animals can be further expanded by activation of osteoblastic cells with parathyroid hormone (PTH) [82], a hormone well-established to expand the HSC pool in a number of murine models [83–86]. These data suggest that OPN restrains the effects of niche activation.

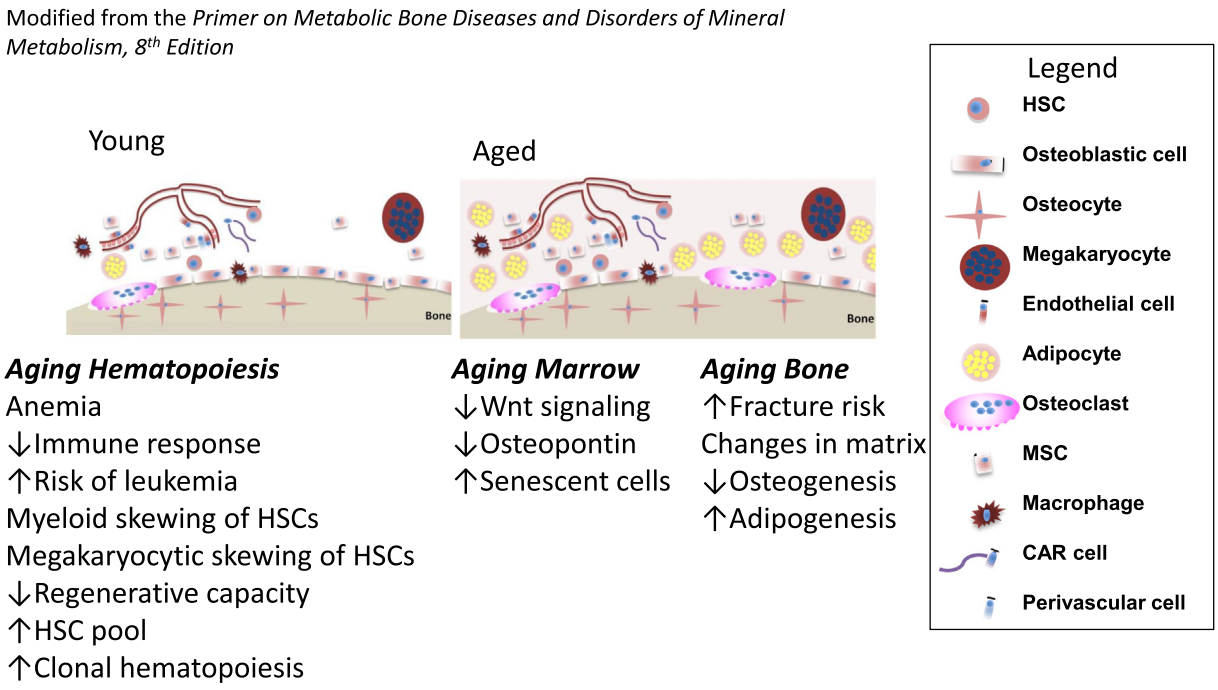

Fig. 1. Impact of aging on bone, marrow and hematopoiesis. Schematic representation of microenvironmental changes identified in the marrow to date. A legend for microenvironmental components is shown. Background color (pink) in aging to highlight the inflammatory/senescent signals recently reported.
Modified from the Primer on Metabolic Bone Diseases and Disorders of Mineral Metabolism, 8th Edition. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Regulation of HSC number by OPN is attributed to different OPN fragments that are formed when OPN is cleaved by the protease thrombin (tc-OPN) [87,88]. Cleavage of OPN by thrombin exposes matricryptic sites that allow HSCs to bind *via* integrins $\alpha_4\beta_1$ or $\alpha_9\beta_1$, facilitating HSC homing and suppressing proliferation. The effect of tc-OPN on downstream signaling pathways in HSCs is currently being explored. The importance of thrombin to this process also implicates megakaryocytes, the principal source of thrombin [87]. Megakaryocytes have been shown to regulate HSCs [89] but also to provide osteoblastic support, since mice with targeted deletion of thrombin in megakaryocytes had increased femoral bone volume [90]. Megakaryocytes had previously been shown to promote murine osteoblastic cells and their support of HSCs after radiation injury [91]. Emerging data showing that calvaria-resident macrophages, also known as osteomacs, are required for the hematopoietic-enhancing activity of osteoblastic cells [92,93], and that megakaryocyte regulate osteomac proliferation, suggesting that interactions of megakaryocytes with osteoblasts are further modulated by osteomacs.

Guidi et al. found that with aging OPN is lost in the bone marrow, specifically by osteoblasts [94]. Incubation of aged HSCs with tc-OPN had a rejuvenating effect [94]. When transplanted, aged HSCs treated with tc-OPN did not show a myeloid bias, compared to vehicle treated HSCs [94]. Of note, aging is associated with many phenotypes seen the OPN-KO: weight gain, altered MSC fate choice, and expanded HSC pool [81,82,94]. MSCs and HSCs are expected to be differentially regulated by OPN, as these stem cell populations interact *via* different integrin heterodimers [81, 87]. These studies underscore the crucial role of an osteoblastic-derived factor that is regulated by megakaryocytes and regulates MSCs and HSC at homeostasis and aging, demonstrating reciprocal interactions of marrow and bone of where the aged microenvironment may be responsible for HSC aging.

4.3. Senescent cells

Cellular senescence is a complex response to stress that results in irreversible arrest of proliferation in cells experiencing potentially transforming conditions, including DNA damage and other cellular stressors [95,96]. When cells undergo senescence, they activate the p53 and RB/p16INK4a pathways, linking DNA damage to this permanent block in the cell cycle [96]. Even though senescent cells are not proliferative, senescent cells are not an inert component of aging tissue, but rather these cells are actively involved in disease pathogenesis. Senescent cells are metabolically active and acquire the senescent-associated secretory phenotype (SASP) [97–99]. The SASP encompasses the release of inflammatory cytokines, chemokines, and proteases into the tissue [97–99]. Work from the Belmonte group showed that premature aging phenotypes, as seen in Werner's syndrome (loss of *WRN gene*), lead to early senescence and acquisition of SASP in induced pluripotent stem cells *in vitro* compared to WT cultures [100]. Senescence in Werner's Syndrome is linked to a loss of heterochromatin structure, further implicating genome maintenance as a regulator of senescence [100]. SASP has been speculated to initially be a beneficial process, which would allow immune recruitment to remove aging cells, and remodel aged tissue [101]. However this inflammation is not cleared and becomes pathological.

Studies using genetic and pharmacologic tools that ablate senescent cells have shown that even a modest reduction in senescent cell populations improves the lifespan and healthspan in murine models [102–105]. Based on these findings, there is increased interest in targeting senescent cells as a means of treating diseases associated with aging [106]. Work from Chang et al. investigated this concept using aged mice and mice that were irradiated in order to induce senescence *in vivo* [106]. In their work, they identify ABT263 (BCL-2/BCL-XL inhibitor) as a senolytic compound that shows preferential toxicity to senescent cells over non-senescent cells *in vitro* [106]. Administration of ABT263 eight weeks after mice had been sub-lethally irradiated (6 Gy) lead to a phenotypic reduction of senescent cells *in vivo*, and an increase in HSC function by transplant [106]. Marrow from ABT263 treated irradiated animals also showed enhanced lymphoid engraftment over myeloid, suggesting that senescent cells can contribute to the myeloid-bias seen in aged HSCs [106]. Treatment of aged animals with ABT263 enhanced their HSCs function, as treated animals had higher

engraftment and a reduction in myeloid-bias [106]. Therefore there is emerging evidence that senescent cells could mediate at least some aspect of the aging phenotype of HSCs.

While Chang et al. did not characterize these senescent cells of the bone marrow, work from the Khosla's laboratory has begun to investigate this [107]. Farr et al. find characteristics of senescence of the bone marrow in both the osteolineage (osteoblasts and osteocytes) and hematopoietic system (CD14+ myeloid cells) [107]. Transcriptionally, both osteolineage and myeloid cells from aged mice show increased expression of the cell cycle inhibitors *p16Ink4a*, *p21*, and *p53* [107]. These transcriptional profiles, including expression of genes associated with senescence, were also increased in human bone biopsies from aged individuals compared to young controls [107]. In addition, transcriptional characterization phenotypically aged osteocytes show telomere dysfunction and exposure of satellite DNA near centromeres, a hallmark of senescent cells [107]. Extending this initial work, using both genetic and pharmacologic approaches, the Khosla laboratory went on to show that senescent cells in the bone marrow are responsible for age-related bone loss [108]. While these data set the stage to implicate the bone marrow microenvironment in HSC aging, additional studies are needed to investigate which senescent cell populations may be primarily responsible for hematopoietic aging.

5. Future directions

Aging of blood and bone is associated with defects in both tissues (Fig. 1), and manifest in dysfunction that results in significant morbidity and mortality. As our understanding of the reciprocal interactions of these tissues deepens, our fields need to further explore the extent of non-cell autonomous contributions to aging, particularly as these cellular relationships may represent valuable and yet currently unexplored targets to ameliorate anemias, susceptibility to infections and malignancies, and to bone fragility.

Acknowledgements

The authors thank members of the Calvi laboratory for discussion and Ms. Susan Daley for administrative support. This work was supported by the National Heart, Lung, and Blood Institute (F31 HL131184 to C.M.H.), the National Institutes of Allergy and Infectious Diseases (U01 AI107276 to L.M.C), the National Cancer Institute (R01 CA166280 to L.M.C), the National Institute on Aging (R01 AG046293 to L.M.C) and funds from the Wilmot Cancer Institute.

References

[1] X. Gao, C. Xu, N. Asada, P.S. Frenette, The hematopoietic stem cell niche: from embryo to adult, Development 145 (2) (2018).
[2] G.M. Crane, E. Jeffery, S.J. Morrison, Adult haematopoietic stem cell niches, Nat. Rev. Immunol. 17 (9) (2017) 573–590.
[3] C. Lopez-Otin, M.A. Blasco, L. Partridge, M. Serrano, G. Kroemer, The hallmarks of aging, Cell 153 (6) (2013) 1194–1217.
[4] C. Beghe, A. Wilson, W.B. Ershler, Prevalence and outcomes of anemia in geriatrics: a systematic review of the literature, Am. J. Med. 116 (Suppl 7A) (2004) 3S–10S.
[5] J.M. Guralnik, R.S. Eisenstaedt, L. Ferrucci, H.G. Klein, R.C. Woodman, Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia, Blood 104 (8) (2004) 2263–2268.
[6] T. Pritz, B. Weinberger, B. Grubeck-Loebenstein, The aging bone marrow and its impact on immune responses in old age, Immunol. Lett. 162 (1 Pt B) (2014) 310–315.
[7] S.S. Chung, C.Y. Park, Aging, hematopoiesis, and the myelodysplastic syndromes, Blood Adv. 1 (26) (2017) 2572–2578.
[8] S. Akunuru, H. Geiger, Aging, clonality, and rejuvenation of hematopoietic stem cells, Trends Mol. Med. 22 (8) (2016) 701–712.
[9] D.J. Rossi, D. Bryder, J.M. Zahn, H. Ahlenius, R. Sonu, A.J. Wagers, I.L. Weissman, Cell intrinsic alterations underlie hematopoietic stem cell aging, Proc. Natl. Acad. Sci. U. S. A. 102 (26) (2005) 9194–9199.
[10] C. Gekas, T. Graf, CD41 expression marks myeloid-biased adult hematopoietic stem cells and increases with age, Blood 121 (22) (2013) 4463–4472.
[11] A. Grover, A. Sanjuan-Pla, S. Thongjuea, J. Carrelha, A. Giustacchini, A. Gambardella, I. Macaulay, E. Mancini, T.C. Luis, A. Mead, S.E. Jacobsen, C. Nerlov, Single-cell RNA sequencing reveals molecular and functional platelet bias of aged haematopoietic stem cells, Nat. Commun. 7 (2016) 11075.
[12] W.W. Pang, E.A. Price, D. Sahoo, I. Beerman, W.J. Maloney, D.J. Rossi, S.L. Schrier, I.L. Weissman, Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age, Proc. Natl. Acad. Sci. U. S. A. 108 (50) (2011) 20012–20017.
[13] D.J. Rossi, D. Bryder, J. Seita, A. Nussenzweig, J. Hoeijmakers, I.L. Weissman, Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age, Nature 447 (7145) (2007) 725–729.
[14] C.E. Rube, A. Fricke, T.A. Widmann, T. Furst, H. Madry, M. Pfreundschuh, C. Rube, Accumulation of DNA damage in hematopoietic stem and progenitor cells during human aging, PLoS One 6 (3) (2011) e17487.
[15] A. Nijnik, L. Woodbine, C. Marchetti, S. Dawson, T. Lambe, C. Liu, N.P. Rodrigues, T.L. Crockford, E. Cabuy, A. Vindigni, T. Enver, J.I. Bell, P. Slijepcevic, C.C. Goodnow, P.A. Jeggo, R.J. Cornall, DNA repair is limiting for haematopoietic stem cells during ageing, Nature 447 (7145) (2007) 686–690.
[16] H. Holstege, W. Pfeiffer, D. Sie, M. Hulsman, T.J. Nicholas, C.C. Lee, T. Ross, J. Lin, M.A. Miller, B. Ylstra, H. Meijers-Heijboer, M.H. Brugman, F.J. Staal, G. Holstege, M.J. Reinders, T.T. Harkins, S. Levy, E.A. Sistermans, Somatic mutations found in the healthy blood compartment of a 115-yr-old woman demonstrate oligoclonal hematopoiesis, Genome Res. 24 (5) (2014) 733–742.
[17] C.J. Gibson, R.C. Lindsley, V. Tchekmedyian, B.G. Mar, J. Shi, S. Jaiswal, A. Bosworth, L. Francisco, J. He, A. Bansal, E.A. Morgan, A.S. Lacasce, A.S. Freedman, D.C. Fisher, E. Jacobsen, P. Armand, E.P. Alyea, J. Koreth, V. Ho, R.J. Soiffer, J.H. Antin, J. Ritz, S. Nikiforow, S.J. Forman, F. Michor, D. Neuberg, R. Bhatia, S. Bhatia, B.L. Ebert, Clonal hematopoiesis associated with adverse outcomes after autologous stem-cell transplantation for lymphoma, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 35 (14) (2017) 1598–1605.
[18] S. Jaiswal, P. Fontanillas, J. Flannick, A. Manning, P.V. Grauman, B.G. Mar, R.C. Lindsley, C.H. Mermel, N. Burtt, A. Chavez, J.M. Higgins, V. Moltchanov, F.C. Kuo, M.J. Kluk, B. Henderson, L. Kinnunen, H.A. Koistinen, C. Ladenvall, G. Getz, A. Correa, B.F. Banahan, S. Gabriel, S. Kathiresan, H.M. Stringham, M.I. McCarthy, M. Boehnke, J. Tuomilehto, C. Haiman, L. Groop, G. Atzmon, J.G. Wilson, D. Neuberg, D. Altshuler, B.L. Ebert, Age-related clonal hematopoiesis associated with adverse outcomes, N. Engl. J. Med. 371 (26) (2014) 2488–2498.
[19] T. McKerrell, N. Park, T. Moreno, C.S. Grove, H. Ponstingl, J. Stephens, C. Crawley, J. Craig, M.A. Scott, C. Hodkinson, J. Baxter, R. Rad, D.R. Forsyth, M.A. Quail, E. Zeggini, W. Ouwehand, I. Varela, G.S. Vassiliou, Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis, Cell Rep. 10 (8) (2015) 1239–1245.
[20] A. Sanjuan-Pla, I.C. Macaulay, C.T. Jensen, P.S. Woll, T.C. Luis, A. Mead, S. Moore, C. Carella, S. Matsuoka, T. Bouriez Jones, O. Chowdhury, L. Stenson, M. Lutteropp, J.C. Green, R. Facchini, H. Boukarabila, A. Grover, A. Gambardella, S. Thongjuea, J. Carrelha, P. Tarrant, D. Atkinson, S.A. Clark, C. Nerlov, S.E. Jacobsen, Platelet-biased stem cells reside at the apex of the haematopoietic stem-cell hierarchy, Nature 502 (7470) (2013) 232–236.
[21] S. Haas, J. Hansson, D. Klimmeck, D. Loeffler, L. Velten, H. Uckelmann, S. Wurzer, A.M. Prendergast, A. Schnell, K. Hexel, R. Santarella-Mellwig, S. Blaszkiewicz, A. Kuck, H. Geiger, M.D. Milsom, L.M. Steinmetz, T. Schroeder, A. Trumpp, J. Krijgsveld, M.A. Essers, Inflammation-induced emergency megakaryopoiesis driven by hematopoietic stem cell-like megakaryocyte progenitors, Cell Stem Cell 17 (4) (2015) 422–434.
[22] J.M. Bernitz, H.S. Kim, B. MacArthur, H. Sieburg, K. Moore, Hematopoietic stem cells count and remember self-renewal divisions, Cell 167 (5) (2016) 1296–1309 (e10).
[23] R. Ceccaldi, K. Parmar, E. Mouly, M. Delord, J.M. Kim, M. Regairaz, M. Pla, N. Vasquez, Q.S. Zhang, C. Pondarre, R. Peffault De Latour, E. Gluckman, M. Cavazzana-Calvo, T. Leblanc, J. Larghero, M. Grompe, G. Socie, A.D. D'Andrea, J. Soulier, Bone marrow failure in Fanconi anemia is triggered by an exacerbated p53/p21 DNA damage response that impairs hematopoietic stem and progenitor cells, Cell Stem Cell 11 (1) (2012) 36–49.
[24] J. Flach, S.T. Bakker, M. Mohrin, P.C. Conroy, E.M. Pietras, D. Reynaud, S. Alvarez, M.E. Diolaiti, F. Ugarte, E.C. Forsberg, M.M. Le Beau, B.A. Stohr, J. Mendez, C.G. Morrison, E. Passegue, Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells, Nature 512 (7513) (2014) 198–202.
[25] I. Beerman, D. Bhattacharya, S. Zandi, M. Sigvardsson, I.L. Weissman, D. Bryder, D.J. Rossi, Functionally distinct hematopoietic stem cells modulate hematopoietic lineage potential during aging by a mechanism of clonal expansion, Proc. Natl. Acad. Sci. U. S. A. 107 (12) (2010) 5465–5470.
[26] K. Sudo, H. Ema, Y. Morita, H. Nakauchi, Age-associated characteristics of murine hematopoietic stem cells, J. Exp. Med. 192 (9) (2000) 1273–1280.
[27] G. de Haan, W. Nijhof, G. Van Zant, Mouse strain-dependent changes in frequency and proliferation of hematopoietic stem cells during aging: correlation between lifespan and cycling activity, Blood 89 (5) (1997) 1543–1550.
[28] J. Chen, C.M. Astle, D.E. Harrison, Development and aging of primitive hematopoietic stem cells in BALB/cBy mice, Exp. Hematol. 27 (5) (1999) 928–935.
[29] Y. Liang, G. Van Zant, S.J. Szilvassy, Effects of aging on the homing and engraftment of murine hematopoietic stem and progenitor cells, Blood 106 (4) (2005) 1479–1487.
[30] L.M. Kamminga, R. van Os, A. Ausema, E.J. Noach, E. Weersing, B. Dontje, E. Vellenga, G. de Haan, Impaired hematopoietic stem cell functioning after serial transplantation and during normal aging, Stem Cells 23 (1) (2005) 82–92.
[31] S.J. Morrison, A.M. Wandycz, K. Akashi, A. Globerson, I.L. Weissman, The aging of hematopoietic stem cells, Nat. Med. 2 (9) (1996) 1011–1016.

[32] C. Kollman, C.W. Howe, C. Anasetti, J.H. Antin, S.M. Davies, A.H. Filipovich, J. Hegland, N. Kamani, N.A. Kernan, R. King, V. Ratanatharathorn, D. Weisdorf, D.L. Confer, Donor characteristics as risk factors in recipients after transplantation of bone marrow from unrelated donors: the effect of donor age, Blood 98 (7) (2001) 2043–2051.
[33] G. Genovese, A.K. Kahler, R.E. Handsaker, J. Lindberg, S.A. Rose, S.F. Bakhoum, K. Chambert, E. Mick, B.M. Neale, M. Fromer, S.M. Purcell, O. Svantesson, M. Landen, M. Hoglund, S. Lehmann, S.B. Gabriel, J.L. Moran, E.S. Lander, P.F. Sullivan, P. Sklar, H. Gronberg, C.M. Hultman, S.A. McCarroll, Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence, N. Engl. J. Med. 371 (26) (2014) 2477–2487.
[34] M. Xie, C. Lu, J. Wang, M.D. McLellan, K.J. Johnson, M.C. Wendl, J.F. McMichael, H.K. Schmidt, V. Yellapantula, C.A. Miller, B.A. Ozenberger, J.S. Welch, D.C. Link, M.J. Walter, E.R. Mardis, J.F. Dipersio, F. Chen, R.K. Wilson, T.J. Ley, L. Ding, Age-related mutations associated with clonal hematopoietic expansion and malignancies, Nat. Med. 20 (12) (2014) 1472–1478.
[35] D.P. Steensma, R. Bejar, S. Jaiswal, R.C. Lindsley, M.A. Sekeres, R.P. Hasserjian, B.L. Ebert, Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes, Blood 126 (1) (2015) 9–16.
[36] S.W. Blume, J.R. Curtis, Medical costs of osteoporosis in the elderly Medicare population, Osteoporos. Int. 22 (6) (2011) 1835–1844.
[37] P.T. Leali, F. Muresu, A. Melis, A. Ruggiu, A. Zachos, C. Doria, Skeletal fragility definition, Clin. Cases Miner. Bone Metab. 8 (2) (2011) 11–13.
[38] A.L. Boskey, E. Donnelly, E. Boskey, L. Spevak, Y. Ma, W. Zhang, J. Lappe, R.R. Recker, Examining the relationships between bone tissue composition, compositional heterogeneity, and fragility fracture: a matched case-controlled FTIRI study, J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 31 (5) (2016) 1070–1081.
[39] A.L. Boskey, D.J. Moore, M. Amling, E. Canalis, A.M. Delany, Infrared analysis of the mineral and matrix in bones of osteonectin-null mice and their wildtype controls, J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 18 (6) (2003) 1005–1011.
[40] D. Farlay, L.A. Armas, E. Gineyts, M.P. Akhter, R.R. Recker, G. Boivin, Nonenzymatic glycation and degree of mineralization are higher in bone from fractured patients with type 1 diabetes mellitus, J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 31 (1) (2016) 190–195.
[41] C.J. Hernandez, S.Y. Tang, B.M. Baumbach, P.B. Hwu, A.N. Sakkee, F. van der Ham, J. DeGroot, R.A. Bank, T.M. Keaveny, Trabecular microfracture and the influence of pyridinium and non-enzymatic glycation-mediated collagen cross-links, Bone 37 (6) (2005) 825–832.
[42] S. Gourion-Arsiquaud, D. Faibish, E. Myers, L. Spevak, J. Compston, A. Hodsman, E. Shane, R.R. Recker, E.R. Boskey, A.L. Boskey, Use of FTIR spectroscopic imaging to identify parameters associated with fragility fracture, J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 24 (9) (2009) 1565–1571.
[43] C.T. Chen, Y.R. Shih, T.K. Kuo, O.K. Lee, Y.H. Wei, Coordinated changes of mitochondrial biogenesis and antioxidant enzymes during osteogenic differentiation of human mesenchymal stem cells, Stem Cells 26 (4) (2008) 960–968 (Dayton, Ohio).
[44] P. Katajisto, J. Dohla, C.L. Chaffer, N. Pentinmikko, N. Marjanovic, S. Iqbal, R. Zoncu, W. Chen, R.A. Weinberg, D.M. Sabatini, cells. Stem, Asymmetric apportioning of aged mitochondria between daughter cells is required for stemness, Science 348 (6232) (2015) 340–343.
[45] C.P. Baines, R.A. Kaiser, N.H. Purcell, N.S. Blair, H. Osinska, M.A. Hambleton, E.W. Brunskill, M.R. Sayen, R.A. Gottlieb, G.W. Dorn, J. Robbins, J.D. Molkentin, Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death, Nature 434 (7033) (2005) 658–662.
[46] L.C. Shum, N.S. White, S.M. Nadtochiy, K.L. Bentley, P.S. Brookes, J.H. Jonason, R.A. Eliseev, Cyclophilin D knock-out mice show enhanced resistance to osteoporosis and to metabolic changes observed in aging bone, PLoS One 11 (5) (2016) e0155709.
[47] O. McGuinness, N. Yafei, A. Costi, M. Crompton, The presence of two classes of high-affinity cyclosporin a binding sites in mitochondria. Evidence that the minor component is involved in the opening of an inner-membrane Ca(2+)-dependent pore, Eur. J. Biochem. 194 (2) (1990) 671–679.
[48] A. Nicolli, E. Basso, V. Petronilli, R.M. Wenger, P. Bernardi, Interactions of cyclophilin with the mitochondrial inner membrane and regulation of the permeability transition pore, and cyclosporin A-sensitive channel, J. Biol. Chem. 271 (4) (1996) 2185–2192.
[49] C. Piot, P. Croisille, P. Staat, H. Thibault, G. Rioufol, N. Mewton, R. Elbelghiti, T.T. Cung, E. Bonnefoy, D. Angoulvant, C. Macia, F. Raczka, C. Sportouch, G. Gahide, G. Finet, X. Andre-Fouet, D. Revel, G. Kirkorian, J.P. Monassier, G. Derumeaux, M. Ovize, Effect of cyclosporine on reperfusion injury in acute myocardial infarction, N. Engl. J. Med. 359 (5) (2008) 473–481.
[50] C.R. Mantel, H.A. O'Leary, B.R. Chitteti, X. Huang, S. Cooper, G. Hangoc, N. Brustovetsky, E.F. Srour, M.R. Lee, S. Messina-Graham, D.M. Haas, N. Falah, R. Kapur, L.M. Pelus, N. Bardeesy, J. Fitamant, M. Ivan, K.S. Kim, H.E. Broxmeyer, Enhancing hematopoietic stem cell transplantation efficacy by mitigating oxygen shock, Cell 161 (7) (2015) 1553–1565.
[51] Y. Omatsu, T. Sugiyama, H. Kohara, G. Kondoh, N. Fujii, K. Kohno, T. Nagasawa, The essential functions of adipo-osteogenic progenitors as the hematopoietic stem and progenitor cell niche, Immunity 33 (3) (2010) 387–399.
[52] C.J. Li, P. Cheng, M.K. Liang, Y.S. Chen, Q. Lu, J.Y. Wang, Z.Y. Xia, H.D. Zhou, X. Cao, H. Xie, E.Y. Liao, X.H. Luo, MicroRNA-188 regulates age-related switch between osteoblast and adipocyte differentiation, J. Clin. Invest. 125 (4) (2015) 1509–1522.
[53] T.K. Chatterjee, G. Idelman, V. Blanco, A.L. Blomkalns, M.G. Piegore Jr., D.S. Weintraub, S. Kumar, S. Rajsheker, D. Manka, S.M. Rudich, Y. Tang, D.Y. Hui, R. Bassel-Duby, E.N. Olson, J.B. Lingrel, S.M. Ho, N.L. Weintraub, Histone deacetylase 9 is a negative regulator of adipogenic differentiation, J. Biol. Chem. 286 (31) (2011) 27836–27847.
[54] B. Sen, Z. Xie, N. Case, W.R. Thompson, G. Uzer, M. Styner, J. Rubin, mTORC2 regulates mechanically induced cytoskeletal reorganization and lineage selection in marrow-derived mesenchymal stem cells, J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 29 (1) (2014) 78–89.
[55] X. Chi, P. Gatti, T. Papoian, Safety of antisense oligonucleotide and siRNA-based therapeutics, Drug Discov. Today 22 (5) (2017) 823–833.
[56] T.H. Ambrosi, A. Scialdone, A. Graja, S. Gohlke, A.M. Jank, C. Bocian, L. Woelk, H. Fan, D.W. Logan, A. Schurmann, L.R. Saraiva, T.J. Schulz, Adipocyte accumulation in the bone marrow during obesity and aging impairs stem cell-based hematopoietic and bone regeneration, Cell Stem Cell 20 (6) (2017 Jun 1) 771–784.
[57] P.K. Fazeli, M.C. Horowitz, O.A. MacDougald, E.L. Scheller, M.S. Rodeheffer, C.J. Rosen, A. Klibanski, Marrow fat and bone–new perspectives, J. Clin. Endocrinol. Metab. 98 (3) (2013) 935–945.
[58] T. Reya, H. Clevers, Wnt signalling in stem cells and cancer, Nature 434 (7035) (2005) 843–850.
[59] T. Reya, A.W. Duncan, L. Ailles, J. Domen, D.C. Scherer, K. Willert, L. Hintz, R. Nusse, I.L. Weissman, A role for Wnt signalling in self-renewal of haematopoietic stem cells, Nature 423 (6938) (2003) 409–414.
[60] W. Goessling, T.E. North, S. Loewer, A.M. Lord, S. Lee, C.L. Stoick-Cooper, G. Weidinger, M. Puder, G.Q. Daley, R.T. Moon, L.I. Zon, Genetic interaction of PGE2 and Wnt signaling regulates developmental specification of stem cells and regeneration, Cell 136 (6) (2009) 1136–1147.
[61] T.E. North, W. Goessling, C.R. Walkley, C. Lengerke, K.R. Kopani, A.M. Lord, G.J. Weber, T.V. Bowman, I.H. Jang, T. Grosser, G.A. Fitzgerald, G.Q. Daley, S.H. Orkin, L.I. Zon, Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis, Nature 447 (7147) (2007) 1007–1011.
[62] T.C. Luis, B.A. Naber, P.P. Roozen, M.H. Brugman, E.F. de Haas, M. Ghazvini, W.E. Fibbe, J.J. van Dongen, R. Fodde, F.J. Staal, Canonical wnt signaling regulates hematopoiesis in a dosage-dependent fashion, Cell Stem Cell 9 (4) (2011) 345–356.
[63] F. Famili, M.H. Brugman, E. Taskesen, B.E. Naber, R. Fodde, F.J. Staal, High levels of canonical Wnt signaling lead to loss of stemness and increased differentiation in hematopoietic stem cells, Stem Cell Rep. 6 (5) (2016) 652–659.
[64] R. Sugimura, X.C. He, A. Venkatraman, F. Arai, A. Box, C. Semerad, J.S. Haug, L. Peng, X.B. Zhong, T. Suda, L. Li, Noncanonical Wnt signaling maintains hematopoietic stem cells in the niche, Cell 150 (2) (2012) 351–365.
[65] M.C. Florian, K.J. Nattamai, K. Dorr, G. Marka, B. Uberle, V. Vas, C. Eckl, I. Andra, M. Schiemann, R.A. Oostendorp, K. Scharffetter-Kochanek, H.A. Kestler, Y. Zheng, H. Geiger, A canonical to non-canonical Wnt signalling switch in haematopoietic stem-cell ageing, Nature 503 (7476) (2013) 392–396.
[66] M.C. Florian, K. Dorr, A. Niebel, D. Daria, H. Schrezenmeier, M. Rojewski, M.D. Filippi, A. Hasenberg, M. Gunzer, K. Scharffetter-Kochanek, Y. Zheng, H. Geiger, Cdc42 activity regulates hematopoietic stem cell aging and rejuvenation, Cell Stem Cell 10 (5) (2012) 520–530.
[67] C.M. Karner, F. Long, Wnt signaling and cellular metabolism in osteoblasts, Cell. Mol. Life Sci. 74 (9) (2017) 1649–1657.
[68] X. Li, Y. Zhang, H. Kang, W. Liu, P. Liu, J. Zhang, S.E. Harris, D. Wu, Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling, J. Biol. Chem. 280 (20) (2005) 19883–19887.
[69] A. Bafico, G. Liu, A. Yaniv, A. Gazit, S.A. Aaronson, Novel mechanism of Wnt signalling inhibition mediated by Dickkopf-1 interaction with LRP6/arrow, Nat. Cell Biol. 3 (7) (2001) 683–686.
[70] Y. Gong, M. Vikkula, L. Boon, J. Liu, P. Beighton, R. Ramesar, L. Peltonen, H. Somer, T. Hirose, B. Dallapiccola, A. De Paepe, W. Swoboda, B. Zabel, A. Superti-Furga, B. Steinmann, H.G. Brunner, A. Jans, R.G. Boles, W. Adkins, M.J. van den Boogaard, B.R. Olsen, M.L. Warman, Osteoporosis-pseudoglioma syndrome, a disorder affecting skeletal strength and vision, is assigned to chromosome region 11q12–13, Am. J. Hum. Genet. 59 (1) (1996) 146–151.
[71] L.M. Boyden, J. Mao, J. Belsky, L. Mitzner, A. Farhi, M.A. Mitnick, D. Wu, K. Insogna, R.P. Lifton, High bone density due to a mutation in LDL-receptor-related protein 5, N. Engl. J. Med. 346 (20) (2002) 1513–1521.
[72] W. Balemans, M. Ebeling, N. Patel, E. Van Hul, P. Olson, M. Dioszegi, C. Lacza, W. Wuyts, J. Van Den Ende, P. Willems, A.F. Paes-Alves, S. Hill, M. Bueno, F.J. Ramos, P. Tacconi, F.G. Dikkers, C. Stratakis, K. Lindpaintner, B. Vickery, D. Foernzler, W. Van Hul, Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST), Hum. Mol. Genet. 10 (5) (2001) 537–543.
[73] W. Balemans, J. Van Den Ende, A. Freire Paes-Alves, F.G. Dikkers, P.J. Willems, F. Vanhoenacker, N. de Almeida-Melo, C.F. Alves, C.A. Stratakis, S.C. Hill, W. Van Hul, Localization of the gene for sclerosteosis to the van Buchem disease-gene region on chromosome 17q12-q21, Am. J. Hum. Genet. 64 (6) (1999) 1661–1669.
[74] M.R. McClung, A. Grauer, S. Boonen, M.A. Bolognese, J.P. Brown, A. Diez-Perez, B.L. Langdahl, J.Y. Reginster, J.R. Zanchetta, S.M. Wasserman, L. Katz, J. Maddox, Y.C. Yang, C. Libanati, H.G. Bone, Romosozumab in postmenopausal women with low bone mineral density, N. Engl. J. Med. 370 (5) (2014) 412–420.
[75] F. Cosman, D.B. Crittenden, J.D. Adachi, N. Binkley, E. Czerwinski, S. Ferrari, L.C. Hofbauer, E. Lau, E.M. Lewiecki, A. Miyauchi, C.A. Zerbini, C.E. Milmont, L. Chen, J. Maddox, P.D. Meisner, C. Libanati, A. Grauer, Romosozumab treatment in postmenopausal women with osteoporosis, N. Engl. J. Med. 375 (16) (2016) 1532–1543.
[76] M. Florio, K. Gunasekaran, M. Stolina, X. Li, L. Liu, B. Tipton, H. Salimi-Moosavi, F.J. Asuncion, C. Li, B. Sun, H.L. Tan, L. Zhang, C.Y. Han, R. Case, A.N. Duguay,

M. Grisanti, J. Stevens, J.K. Pretorius, E. Pacheco, H. Jones, Q. Chen, B.D. Soriano, J. Wen, B. Heron, F.W. Jacobsen, E. Brisan, W.G. Richards, H.Z. Ke, M.S. Ominsky, A bispecific antibody targeting sclerostin and DKK-1 promotes bone mass accrual and fracture repair, Nat. Commun. 7 (2016) 11505.

[77] R.R. Recker, C.T. Benson, T. Matsumoto, M.A. Bolognese, D.A. Robins, J. Alam, A.Y. Chiang, L. Hu, J.H. Krege, H. Sowa, B.H. Mitlak, S.L. Myers, A randomized, double-blind phase 2 clinical trial of blosozumab, a sclerostin antibody, in postmenopausal women with low bone mineral density, J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 30 (2) (2015) 216–224.

[78] C.P. Recknor, R.R. Recker, C.T. Benson, D.A. Robins, A.Y. Chiang, J. Alam, L. Hu, T. Matsumoto, H. Sowa, J.H. Sloan, R.J. Konrad, B.H. Mitlak, A.A. Sipos, The effect of discontinuing treatment with blosozumab: follow-up results of a phase 2 randomized clinical trial in postmenopausal women with low bone mineral density, J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 30 (9) (2015) 1717–1725.

[79] C.M. Laine, K.S. Joeng, P.M. Campeau, R. Kiviranta, K. Tarkkonen, M. Grover, J.T. Lu, M. Pekkinen, M. Wessman, T.J. Heino, V. Nieminen-Pihala, M. Aronen, T. Laine, H. Kroger, W.G. Cole, A.E. Lehesjoki, L. Nevarez, D. Krakow, C.J. Curry, D.H. Cohn, R.A. Gibbs, B.H. Lee, O. Makitie, WNT1 mutations in early-onset osteoporosis and osteogenesis imperfecta, N. Engl. J. Med. 368 (19) (2013) 1809–1816.

[80] D.N. Haylock, S.K. Nilsson, Osteopontin: a bridge between bone and blood, Br. J. Haematol. 134 (5) (2006) 467–474.

[81] Q. Chen, P. Shou, L. Zhang, C. Xu, C. Zheng, Y. Han, W. Li, Y. Huang, X. Zhang, C. Shao, A.I. Roberts, A.B. Rabson, G. Ren, Y. Zhang, Y. Wang, D.T. Denhardt, Y. Shi, An osteopontin-integrin interaction plays a critical role in directing adipogenesis and osteogenesis by mesenchymal stem cells, Stem Cells 32 (2) (2014) 327–337 Dayton, Ohio.

[82] S. Stier, Y. Ko, R. Forkert, C. Lutz, T. Neuhaus, E. Grunewald, T. Cheng, D. Dombkowski, L.M. Calvi, S.R. Rittling, D.T. Scadden, Osteopontin is a hematopoietic stem cell niche component that negatively regulates stem cell pool size, J. Exp. Med. 201 (11) (2005) 1781–1791.

[83] L.M. Calvi, G.B. Adams, K.W. Weibrecht, J.M. Weber, D.P. Olson, M.C. Knight, R.P. Martin, E. Schipani, P. Divieti, F.R. Bringhurst, L.A. Milner, H.M. Kronenberg, D.T. Scadden, Osteoblastic cells regulate the haematopoietic stem cell niche, Nature 425 (6960) (2003) 841–846.

[84] G.B. Adams, R.P. Martin, I.R. Alley, K.T. Chabner, K.S. Cohen, L.M. Calvi, H.M. Kronenberg, D.T. Scadden, Therapeutic targeting of a stem cell niche (vol 25, pg 238, 2007), Nat. Biotechnol. 25 (2) (2007 Feb) 238–243.

[85] T. Itkin, K.B. Kaufmann, S. Gur-Cohen, A. Ludin, T. Lapidot, Fibroblast growth factor signaling promotes physiological bone remodeling and stem cell self-renewal, Curr. Opin. Hematol. 20 (3) (2013) 237–244.

[86] D.S. Krause, K. Fulzele, A. Catic, M. Hurley, S. Lezeau, R.P. Hasserjian, J. Wu, P. Divieti-Pajevich, R.A. Van Etten, E. Schipani, D.T. Scadden, Oral Presentations: Concurrent Oral Session, Vol. 39 Cancer and Bone American Society for Hematology, San Diego, 2011.

[87] M.J. Storan, S.Y. Heazlewood, C.K. Heazlewood, D.N. Haylock, W.S. Alexander, R.J. Neaves, A. Oteiza, S.K. Nilsson, Brief report: factors released by megakaryocytes thrombin cleave osteopontin to negatively regulate hematopoietic stem cells, Stem Cells 33 (7) (2015) 2351–2357.

[88] J. Grassinger, D.N. Haylock, M.J. Storan, G.O. Haines, B. Williams, G.A. Whitty, A.R. Vinson, C.L. Be, S. Li, E.S. Sorensen, P.P. Tam, D.T. Denhardt, D. Sheppard, P.F. Choong, S.K. Nilsson, Thrombin-cleaved osteopontin regulates hemopoietic stem and progenitor cell functions through interactions with alpha9beta1 and alpha4beta1 integrins, Blood 114 (1) (2009) 49–59.

[89] I. Bruns, D. Lucas, S. Pinho, J. Ahmed, M.P. Lambert, Y. Kunisaki, C. Scheiermann, L. Schiff, M. Poncz, A. Bergman, P.S. Frenette, Megakaryocytes regulate hematopoietic stem cell quiescence through CXCL4 secretion, Nat. Med. 20 (11) (2014) 1315–1320.

[90] M.B. Alvarez, L. Xu, P. Childress, K.A. Maupin, S.F.G. Mohamad, B. Chitteti, E. Himes, D.J. Olivos Iii, Y.H. Cheng, S.J. Conway, E.F. Srour, M.A. Kacena, Megakaryocyte and osteoblast interactions modulate bone mass and hematopoiesis, Stem Cells Dev. 27 (10) (2018 May 15) 671–682.

[91] T.S. Olson, A. Caselli, S. Otsuru, T.J. Hofmann, R. Williams, P. Paolucci, M. Dominici, E.M. Horwitz, Megakaryocytes promote murine osteoblastic HSC niche expansion and stem cell engraftment after radioablative conditioning, Blood 121 (26) (2013) 5238–5249.

[92] S.F. Mohamad, L. Xu, J. Ghosh, P.J. Childress, I. Abeysekera, E.R. Himes, H. Wu, M.B. Alvarez, K.M. Davis, A. Aguilar-Perez, J.M. Hong, A. Bruzzaniti, M.A. Kacena, E.F. Srour, Osteomacs interact with megakaryocytes and osteoblasts to regulate murine hematopoietic stem cell function, Blood Adv. 1 (26) (2017) 2520–2528.

[93] M. Zhao, J.M. Perry, H. Marshall, A. Venkatraman, P. Qian, X.C. He, J. Ahamed, L. Li, Megakaryocytes maintain homeostatic quiescence and promote post-injury regeneration of hematopoietic stem cells, Nat. Med. 20 (11) (2014) 1321–1326.

[94] N. Guidi, M. Sacma, L. Standker, K. Soller, G. Marka, K. Eiwen, J.M. Weiss, F. Kirchhoff, T. Weil, J.A. Cancelas, M.C. Florian, H. Geiger, Osteopontin attenuates aging-associated phenotypes of hematopoietic, Stem Cells 36 (7) (2017) 840–853.

[95] T. Tchkonia, Y. Zhu, J. van Deursen, J. Campisi, J.L. Kirkland, Cellular senescence and the senescent secretory phenotype: therapeutic opportunities, J. Clin. Invest. 123 (3) (2013) 966–972.

[96] C.D. Wiley, J. Campisi, From ancient pathways to aging cells-connecting metabolism and cellular senescence, Cell Metab. 23 (6) (2016) 1013–1021.

[97] J.P. Coppe, C.K. Patil, F. Rodier, A. Krtolica, C.M. Beausejour, S. Parrinello, J.G. Hodgson, K. Chin, P.Y. Desprez, J. Campisi, A human-like senescence-associated secretory phenotype is conserved in mouse cells dependent on physiological oxygen, PLoS One 5 (2) (2010) e9188.

[98] J.P. Coppe, C.K. Patil, F. Rodier, Y. Sun, D.P. Munoz, J. Goldstein, P.S. Nelson, P.Y. Desprez, J. Campisi, Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor, PLoS Biol. 6 (12) (2008) 2853–2868.

[99] T. Kuilman, C. Michaloglou, L.C. Vredeveld, S. Douma, R. van Doorn, C.J. Desmet, L.A. Aarden, W.J. Mooi, D.S. Peeper, Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network, Cell 133 (6) (2008) 1019–1031.

[100] W. Zhang, J. Li, K. Suzuki, J. Qu, P. Wang, J. Zhou, X. Liu, R. Ren, X. Xu, A. Ocampo, T. Yuan, J. Yang, Y. Li, L. Shi, D. Guan, H. Pan, S. Duan, Z. Ding, M. Li, F. Yi, R. Bai, Y. Wang, C. Chen, F. Yang, X. Li, Z. Wang, E. Aizawa, A. Goebl, R.D. Soligalla, P. Reddy, C.R. Esteban, F. Tang, G.H. Liu, J.C. Belmonte, Aging stem cells. A Werner syndrome stem cell model unveils heterochromatin alterations as a driver of human aging, Science 348 (6239) (2015) 1160–1163.

[101] B.G. Childs, M. Durik, D.J. Baker, J.M. van Deursen, Cellular senescence in aging and age-related disease: from mechanisms to therapy, Nat. Med. 21 (12) (2015) 1424–1435.

[102] M. Demaria, N. Ohtani, S.A. Youssef, F. Rodier, W. Toussaint, J.R. Mitchell, R.M. Laberge, J. Vijg, H. Van Steeg, M.E. Dolle, J.H. Hoeijmakers, A. de Bruin, E. Hara, J. Campisi, An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA, Dev. Cell 31 (6) (2014) 722–733.

[103] D.J. Baker, T. Wijshake, T. Tchkonia, N.K. LeBrasseur, B.G. Childs, B. van de Sluis, J.L. Kirkland, J.M. van Deursen, Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders, Nature 479 (7372) (2011) 232–236.

[104] M. Xu, A.K. Palmer, H. Ding, M.M. Weivoda, T. Pirtskhalava, T.A. White, A. Sepe, K.O. Johnson, M.B. Stout, N. Giorgadze, M.D. Jensen, N.K. LeBrasseur, T. Tchkonia, J.L. Kirkland, Targeting senescent cells enhances adipogenesis and metabolic function in old age, elife 4 (2015) e12997.

[105] C.M. Roos, B. Zhang, A.K. Palmer, M.B. Ogrodnik, T. Pirtskhalava, N.M. Thalji, M. Hagler, D. Jurk, L.A. Smith, G. Casaclang-Verzosa, Y. Zhu, M.J. Schafer, T. Tchkonia, J.L. Kirkland, J.D. Miller, Chronic senolytic treatment alleviates established vasomotor dysfunction in aged or atherosclerotic mice, Aging Cell 15 (5) (2016) 973–977.

[106] J. Chang, Y. Wang, L. Shao, R.M. Laberge, M. Demaria, J. Campisi, K. Janakiraman, N.E. Sharpless, S. Ding, W. Feng, Y. Luo, X. Wang, N. Aykin-Burns, K. Krager, U. Ponnappan, M. Hauer-Jensen, A. Meng, D. Zhou, Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice, Nat. Med. 22 (1) (2016) 78–83.

[107] J.N. Farr, D.G. Fraser, H. Wang, K. Jaehn, M.B. Ogrodnik, M.M. Weivoda, M.T. Drake, T. Tchkonia, N.K. LeBrasseur, J.L. Kirkland, L.F. Bonewald, R.J. Pignolo, D.G. Monroe, S. Khosla, Identification of senescent cells in the bone microenvironment, J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 31 (11) (2016) 1920–1929.

[108] J.N. Farr, M. Xu, M.M. Weivoda, D.G. Monroe, D.G. Fraser, J.L. Onken, B.A. Negley, J.G. Sfeir, M.B. Ogrodnik, C.M. Hachfeld, N.K. LeBrasseur, M.T. Drake, R.J. Pignolo, T. Pirtskhalava, T. Tchkonia, M.J. Oursler, J.L. Kirkland, S. Khosla, Targeting cellular senescence prevents age-related bone loss in mice, Nat. Med. 23 (9) (2017) 1072–1079.