The ageing haematopoietic stem cell compartment

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Abstract | Stem cell ageing underlies the ageing of tissues, especially those with a high cellular turnover. There is growing evidence that the ageing of the immune system is initiated at the very top of the haematopoietic hierarchy and that the ageing of haematopoietic stem cells (HSCs) directly contributes to changes in the immune system, referred to as immunosenescence. In this Review, we summarize the phenotypes of ageing HSCs and discuss how the cell-intrinsic and cell-extrinsic mechanisms of HSC ageing might promote immunosenescence. Stem cell ageing has long been considered to be irreversible. However, recent findings indicate that several molecular pathways could be targeted to rejuvenate HSCs and thus to reverse some aspects of immunosenescence.

HSC niche
A specialized microenvironment that interacts with haematopoietic stem cells (HSCs) to regulate their fate.

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The current demographic shift towards an ageing population is an unprecedented global phenomenon that has profound implications. Ageing is associated with tissue attrition and an increased incidence of many types of cancers, including both myeloid and lymphoid leukaemias, and other haematopoietic cell malignancies[1,2]. Thus, we need to understand the molecular and cellular mechanisms of ageing to attenuate ageing-associated tissue attrition and disease, and to improve the quality of life for the elderly.

Alterations that affect the immune system — globally referred to as immunosenescence — are among the factors associated with ageing that reduce the quality of life for the elderly. Age-related changes in the immune system contribute to the increased susceptibility of the elderly to infectious diseases, autoimmunity, anaemia, vaccine failure and, possibly, cancers such as leukaemia[2–5]. Many of these ageing-related pathologies can be attributed to the ageing of the adaptive immune system. For example, the peripheral B cell pool fills up with memory cells in elderly individuals, and this results in limited diversity in the B cell repertoire, reduced effectiveness of vaccination, and an age-related increase in circulating autoreactive antibodies[3,4]. The number of naive T cells decreases considerably with age, which is partly linked to the involution of the thymus[6,7]. The defective generation of naive T cells, and the accumulation of effector and memory T cells, results in decreased diversity in the T cell repertoire after the age of 70 (REFS 8,9). As changes in the T cell compartment precede the ageing-associated changes in the B cell compartment, it is thought that T cells fail to sustain B cell proliferation and high-affinity antibody production in elderly individuals[10,11].

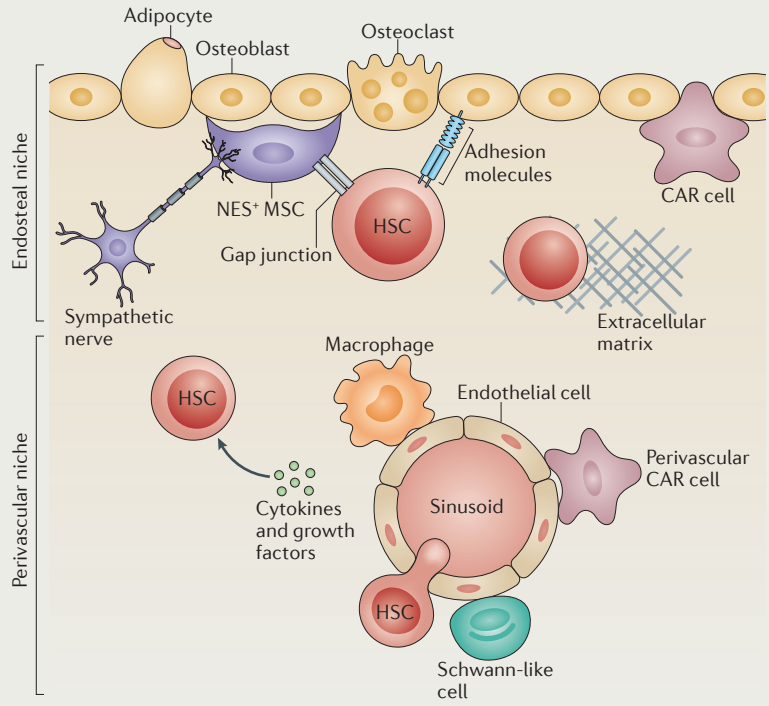
The innate immune system is also affected by ageing. Although an increase in the number of myeloid precursors has been described in the bone marrow of elderly people, the oxidative burst and the phagocytic capacity of both macrophages and neutrophils are decreased in these individuals[12,13]. Moreover, the levels of soluble immune mediators are altered with ageing. Elevated plasma concentrations of several cytokines, such as interleukin-6 (IL-6), tumour necrosis factor and IL-1β, have been described as predictive markers for morbidity and mortality in the elderly. These factors are thought to result in an ageing-associated subclinical inflammatory status, which has also been described as 'inflamm-ageing' and drives the development and progression of age-related diseases such as osteoporosis and neurodegenerative disorders[14].

The dysfunction of haematopoietic stem cells (HSCs), which give rise to both myeloid and lymphoid lineages, might underlie the ageing of the innate and adaptive immune systems. Indeed, there is now growing evidence that the ageing of the immune system is initiated at the very top of the haematopoietic hierarchy. For example, B cell lymphopoiesis is directly affected by HSC ageing, and an ageing-associated reduction in the generation of B cell precursors has been linked to impaired function of aged HSCs[12,15,16].

In adults, HSCs reside primarily in the bone marrow within a specialized microenvironment called the HSC niche. The HSC niche provides soluble factors and cell–cell interactions that are crucial for regulating HSC self-renewal and differentiation (BOX 1). In the adult bone marrow there is a low frequency of HSCs, with two to five HSCs per 10^5 total bone marrow cells[17,18].

Box 1 | **The HSC niche**

The bone marrow microenvironment provides regions that support the function of haematopoietic stem cells (HSCs) and other types of haematopoietic cells, including monocytes and lymphocytes. Cells within these regions constitute the HSC niche[116] (see figure). The HSC niche contributes to the control of HSC quiescence, proliferation, self-renewal and differentiation. The niche within the bone consists of a network of vessels, nerve fibres, bone remodelling cells and subpopulations of haematopoietic cells, which result in a complex three-dimensional architecture[44,46,100,135–137]. These niche functions are mediated by crosstalk between niche cells and HSCs. Novel research indicates that this signal exchange is multidirectional and multidimensional[136]. Signals between the niche cells and HSCs can be transmitted via direct cell–cell contact (through adhesion receptors[46] and gap junctions such as connexin 43 junctions)[109–111], cytokines and chemokines (such as CXC-chemokine ligand 12 (CXCL12; also known as SDF1))[138], growth factors and via components of the extracellular matrix (such as fibronectin). HSCs often reside close to the endosteum (the endosteal niche) and the vascular system (the vascular niche) in the bone marrow. In these niches, HSCs are in close proximity to reticular cells expressing high levels of CXCL12 (CXCL12-abundant reticular cells; CAR cells), Schwann-like cells and nestin-positive mesenchymal stem cells (NES$^+$ MSCs), which are all closely surrounded by nerve fibres[139]. Niches comprise non-haematopoietic cells, such as endothelial cells, osteoblasts and osteoclasts, adipocytes and MSCs, but haematopoietic cells (mainly macrophages) also contribute to niche function[140].



In this Review, we summarize the phenotypes of ageing HSCs and discuss current concepts in HSC ageing and its consequences for haematopoiesis. We also describe the cell-intrinsic and cell-extrinsic pathways that contribute to the ageing of HSCs and how these might influence immunosenescence. It is generally accepted that stem cell ageing is the primary factor that drives the ageing of tissues, and, importantly, underlies the ageing of biological systems that are characterized by a high cellular turnover, such as the immune system. Although stem cell ageing has long been considered to be irreversible, we discuss possible therapeutic approaches to rejuvenate HSCs or to attenuate their ageing with a view to reversing or preventing immunosenescence.

Self-renewal
The capacity to recreate stem cells with differentiation potentials that are similar to those of the mother stem cell. Self-renewal can result from either an asymmetric cell division that yields a daughter stem cell and a cell committed to differentiation, or from a symmetric cell division that yields two daughter stem cells. Alternatively, stem cell differentiation can result in the loss of self-renewal capacity.

What separates young from aged HSCs?

There are several generally accepted functional, cellular and molecular criteria that can be used to distinguish between young and aged HSCs. In this section, we summarize and interpret the HSC phenotypes that are associated with ageing and suggest a set of criteria that should be used to define the phenotype of aged HSCs (FIG. 1). As ageing is a gradual process, ageing-associated phenotypes are sometimes ambiguous; although some distinct phenotypes can help to clearly distinguish between young and aged HSCs, there is also a range of phenotypes on the basis of which HSCs cannot always be easily classified as young or aged.

Increased HSC numbers and decreased regenerative potential. Stem cells are often considered to be a 'fountain of eternal youth' because of their capacity for self-renewal and their differentiation potential. So, it seems likely that loss of stem cell activity underlies ageing-associated tissue attrition.

Research performed over the past few decades now enables HSCs to be phenotypically identified on the basis of several sets of cell surface markers, in both humans and mice (TABLE 1). Unexpectedly, the number of such phenotypically defined HSCs in the bone marrow increases by two- to tenfold with ageing, regardless of which distinct sets of cell surface markers are used to define HSCs, in both mice and humans[19–26] (TABLE 1). Intuitively, the increased HSC numbers that are observed in elderly individuals are considered to be beneficial, as these HSCs might, for example, sustain immune functions. Nevertheless, under conditions of stress and regeneration, as in serial transplantation assays, aged HSCs exhibit several functional defects, including a diminished regenerative potential, as a result of their reduced long-term self-renewal capacity. The increase in the number of HSCs does not compensate for their loss in function, and this leads to an overall reduction in the regenerative capacity of the pool of aged HSCs[27] (FIG. 1). This is particularly evident when purified stem cell populations are analysed[25,28].

The mechanisms that underlie the ageing-associated increase in HSC numbers are still poorly understood, even though this aspect of HSC biology has been known for a long time. It has been proposed that the ageing-associated increase in HSC numbers is a compensatory mechanism to overcome their reported loss in function. However, this hypothesis is challenged by the fact that both aged and young HSCs have a comparable frequency of cell divisions[25]. Alternatively, an increase in the frequency of self-renewing symmetric cell divisions might contribute to the high numbers and impaired function of aged HSCs[27]. In addition, a loss in responsiveness to extrinsic cues or age-related changes in the expression and secretion of systemic and niche factors[29,30] have been suggested to contribute to the increased numbers and impaired function of aged HSCs. However, in mice, the increase in HSC numbers can be recapitulated by the transplantation of aged HSCs into young recipients[19,25]. Therefore, there is a consensus among laboratories studying the ageing

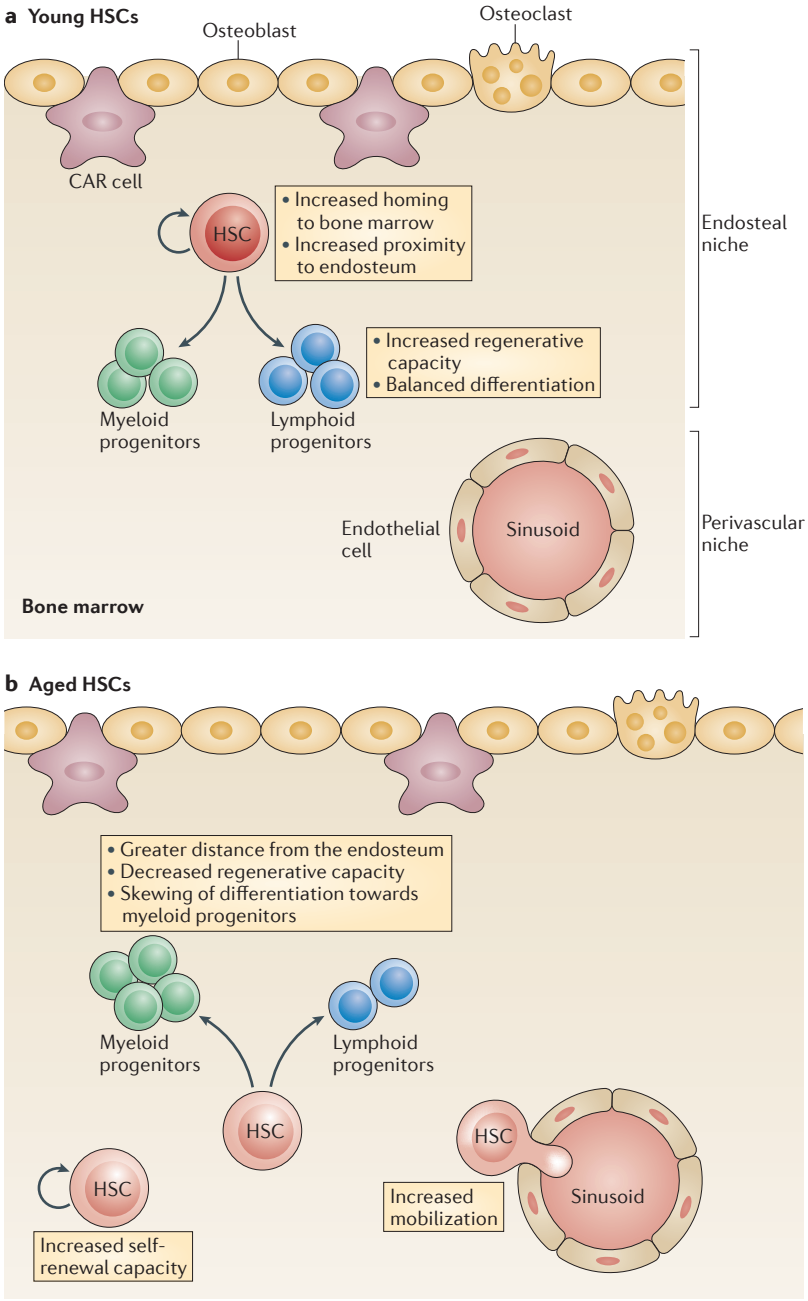


Figure 1 | **Phenotypical and functional changes in HSCs upon ageing. a** | Young haematopoietic stem cells (HSCs) home to the bone marrow and localize in close proximity to the endosteum. They have high self-renewal and regenerative capacities, and a balanced differentiation potential towards lymphoid and myeloid progenitor cells. **b** | The number of phenotypic HSCs in the bone marrow increases with ageing, and this is probably a consequence of an enhanced self-renewal activity of aged HSCs, even though the regenerative capacity (serial transplantability) of individual aged stem cells is reduced compared to young HSCs. The localization of aged HSCs in the bone marrow is different to that of young HSCs; aged HSCs localize away from the endosteal stem cell niche following their transplantation. This implies that aged HSCs select for niches that are distinct from those that young HSCs occupy. Another hallmark of aged HSCs is that following their transfer into recipient mice they exhibit about a two-fold reduced ability to home to the bone marrow compared with young HSCs (not shown). Moreover, aged HSCs can be mobilized in higher numbers than young HSCs in response to cytokine stimulation. Finally, a central hallmark of aged HSCs is their skewing towards myeloid cell differentiation, as they provide more myeloid progenitor cells and fewer lymphoid progenitor cells compared with young HSCs. CAR, CXC-chemokine ligand 12-abundant reticular.

of HSCs that it is primarily cell-intrinsic mechanisms that drive the changes in HSC numbers during ageing and that cell-extrinsic factors have only a limited role in this HSC ageing phenotype.

Skewed differentiation potential. Distinct hallmarks of the ageing haematopoietic system include the onset of anaemia, decreased competence of the adaptive immune system and an expansion of myeloid cells[1]. HSC ageing has been implicated in this skewing of haematopoietic cell differentiation, although multiple events taking place downstream of HSC differentiation, such as the involution of the thymus, might also contribute to this phenotype[31]. Aged HSCs exhibit a markedly decreased output of cells of the lymphoid and erythroid lineages, whereas the myeloid lineage output of aged HSCs is maintained or even increased compared with young HSCs[23,25] (FIG. 1). Despite their increased numbers, the quality of the myeloid cells produced by aged HSCs is compromised[32], which is similar to what is observed with HSCs themselves. Taken together, these aspects of immunosenescence are at least partially responsible for the increased prevalence of infectious diseases and the low efficacy of vaccination in the elderly[3].

Lineage specification occurs during the early stages of haematopoiesis, either following the differentiation of HSCs into common lymphoid progenitor (CLP) cells and common myeloid progenitor (CMP) cells via one or more intermediate steps, as has been suggested by several different models[1], or following the separation of cells with megakaryocyte–erythroid potential from those with granulocyte–macrophage, B cell and T cell potential[33]. Ageing-associated haematopoietic cell lineage skewing seems to involve the disturbance of these early HSC differentiation steps, as aged mice have increased numbers of CMP cells and decreased numbers of CLP cells compared with young mice[19]. This lineage skewing is evident both in the steady state and after the transplantation of aged HSCs into young recipient mice[19,25]. Thus, this ageing-related HSC phenotype also seems to be primarily stem cell-intrinsic. Elderly individuals also have reduced numbers of both CLP cells and early B lymphoid progenitor cells but, contrary to what has been observed in mice, this reduction in the number of CLP cells is not accompanied by an increase in the CMP compartment[12,34].

The early lymphoid to myeloid lineage skewing might be caused by changes in HSC differentiation or by the altered proliferation or survival of CMP cells and CLP cells. It has become clear that there is considerable functional heterogeneity among the most primitive HSCs in terms of their differentiation potentials: some HSCs show a relatively low capacity to differentiate into lymphoid cells and can be regarded as myeloid-biased, some HSCs show the opposite behaviour and can be regarded as lymphoid-biased, whereas other HSCs seem to maintain a balanced output and are regarded as balanced HSCs[35–38]. Consequently, an alternative explanation for ageing-induced lineage skewing is that the differentiation potential of individual HSCs does not change

Table 1 | **Changes in mouse and human HSC phenotypes upon ageing**

HSC population	Changes upon ageing
Mouse	
$LIN^-THY1^-SCA1^+$ (REF. 148)	Not known
$LIN^-SCA1^+KIT^+CD34^-$ (REF. 149)	Not known
Side population (high Hoechst efflux) $LIN^-SCA1^+KIT^+$ (REF. 150)	Expansion of $LIN^-SCA1^+KIT^+$ cells with high Hoechst efflux activity* (myeloid-biased)[40]
$LIN^-SCA1^+KIT^+CD34^-$ cells with the highest Hoechst efflux activity‡ (REF. 151)	Not known
$LIN^-SCA1^+KIT^+CD34^-FLK2^-$ (REF. 152) (LT-HSCs)	Increased frequency of $LIN^-SCA1^+KIT^+CD34^-FLK2^-$ cells (LT-HSCs) and decreased frequency of $LIN^-SCA1^+KIT^+CD34^+FLK2^+$cells (lymphoid committed progenitors)[19,25]
$CD244^-CD48^-CD150^+$ (REF. 153)	Not known
$LIN^-SCA1^+KIT^+CD34^-FLK2^-CD48^-CD41^-CD150^+$ (REF. 154)	Increased frequency of $LIN^-SCA1^+KIT^+CD34^-FLK2^-CD48^-CD41^-CD150^{hi}$ cells (myeloid-biased LT-HSCs)[24]
$CD45^+EPCR^+CD48^-CD150^+$ (REF. 18)	Not known
$LIN^-SCA1^+KIT^+CD48^-CD150^+EPCR^+CD34^-$ (REF. 28)	Increased frequency of $LIN^-SCA1^+KIT^+CD48^-CD150^+EPCR^+CD34^-$ cells[28]
Human	
$LIN^-CD34^+CD38^-CD45RA^-CD90^+$ (REF. 155)	Increased frequency of $LIN^-CD34^+CD38^-$cells and of $LIN^-CD34^+CD38^-CD45RA^-CD90^+$ cells[12,34]
$LIN^-CD34^+CD38^-CD45RA^-CD90^+RHO^{low}CD49F^+$ (REF. 156)	Not known

EPCR, endothelial protein C receptor; FLK2, fetal liver kinase 2; HSC, haematopoietic stem cell; LIN, lineage; LT-HSC, long-term HSC; SCA1, stem cell antigen 1. *Also described as side population, as determined by Hoechst blue versus Hoechst red staining. ‡Also described as tip of side population, as determined by Hoechst blue versus Hoechst red staining.

Serial transplantation assays
Assays used to test the ability of haematopoietic stem cells (HSCs) to undergo self-renewal. The number of serial transplantations that the original donor's bone marrow can perform successfully is a measure of the self-renewal capacity of the HSCs it contains. The serial transplantation of defined numbers of purified HSCs allows the normalization of differences in stem cell frequencies among mice of different ages and offers a more controlled 'per cell' readout than serial transplantation of unfractionated bone marrow cells.

Symmetric cell divisions
Stem cell divisions in which the two daughter cells are identical with respect to function and differentiation potential.

Common lymphoid progenitor
(CLP). A progenitor that is committed to the lymphoid lineage and can give rise to all lymphocyte subsets, including T cells, B cells and natural killer cells. CLP cells are defined as $FLK2^+IL\text{-}7R\alpha^+KIT^{low/hi}SCA1^{low/hi}LIN^-$ in mice, and $LIN^-CD34^+CD38^+CD127^+$ in humans.

Common myeloid progenitor
(CMP). A progenitor that is committed to the myeloid lineage and can give rise to all myeloid subsets, including neutrophils, basophils, monocytes and platelets. CMP cells are defined as $LIN^-IL\text{-}7R\alpha^-SCA1^-KIT^+FcRI^{low}CD34^+$ in mice and $LIN^-CD34^+CD38^+CD123^+CD45RA^-$ in humans.

Bone marrow homing
The process whereby haematopoietic stem cells (HSCs) migrate from the circulation to the HSC niche. Homing is distinct from lodgement, which is the ability of HSCs to enter the niche and stay there, as well as from engraftment, which is the ability of HSCs to respond to appropriate maintenance and differentiation signals when they are lodged.

with age; instead, the composition of the HSC pool is altered. This hypothesis is supported by data showing that in mice the number of myeloid-biased HSC clones is increased compared with the number of lymphoid-biased or balanced clones upon ageing[24,39–41]. Notably, clonal analyses of HSCs that had been isolated from young and aged mice revealed that there are higher numbers of myeloid-biased HSCs in the aged bone marrow compared with the young bone marrow, and that all aged HSC subtypes possess a reduced proliferative capacity as well as additional functional deficiencies, including reduced bone marrow homing *in vivo*.

These observations demonstrate that all HSC subtypes age[28], indicating that a combination of both mechanisms — that is, changes in the composition of the HSC pool as well as changes in individual HSCs — cause lineage skewing. Finally, challenging the concept that cell-intrinsic mechanisms are involved in lineage skewing, recent exciting publications have identified a crucial role for environmental (HSC-extrinsic) factors in the establishment of the ageing-associated myeloid lineage skewing phenotype in mice[42,43]. It is therefore probable that a complex network of primarily HSC-intrinsic but also HSC-extrinsic factors affect the skewing of HSC differentiation in aged mammals.

Altered homing to, and mobilization from, the bone marrow. HSC niches comprise multiple cell types (BOX 1) and promote HSC maintenance by regulating HSC self-renewal, quiescence, mobilization and differentiation[44–46]. Recent reports indicate that young and aged HSCs have distinct niche selectivities *in vivo*, as suggested by the distinct positions of young and aged HSCs relative to the endosteum[25,47] and by the impaired adhesive properties of aged HSCs *in vitro*. Indeed, the recruitment of HSCs to the blood in response to several stimuli (including treatment with chemotherapy or cytokines) — a process termed mobilization — is increased in aged HSCs compared with young HSCs[25,28,48–50] (FIG. 1). Taken together, these observations highlight the differences either in the intrinsic adhesive properties of aged HSCs or in the properties of the niche cells that interact with HSCs in the aged bone marrow[49]. There is a lack of reliable data on changes in stem cell niche interactions upon ageing, probably because of the difficulties associated with experimentally determining these complex interactions *in vivo*. A better characterization of the expression pattern of adhesion molecules on aged HSCs[48] might help to explain how the crosstalk between HSCs and their niche changes upon ageing.

Towards a generally accepted phenotype of aged HSCs. An increasing number of phenotypes have recently been associated with the physiological ageing HSCs. The criteria used to identify HSC ageing include: an increased numbers of cells with an HSC-associated cell surface phenotype; a reduced capacity of HSCs for self-renewal, as determined by serial transplantation assays *in vivo* or by proliferation assays *in vitro*; lineage skewing upon transplantation of HSCs *in vivo*; enhanced mobilization of HSCs from the bone marrow into the blood; and reduced homing of HSCs back to the bone marrow, with

those that do home back showing a distinct niche selectivity (FIG. 1). Moreover, exciting novel approaches have shown that, in addition to HSC-intrinsic phenotypes, HSC–niche interactions change with ageing. These criteria might help to distinguish the 'physiological ageing phenotype' of HSCs from HSC phenotypes that only partially correlate with ageing.

Cell-intrinsic mechanisms involved in HSC ageing

In this section we review and discuss HSC-intrinsic mechanisms that are likely to be involved in HSC ageing (FIG. 2). Transcriptome profiles from aged and young HSCs have been used to identify genes that correlate with HSC ageing and to characterize the possible molecular mechanisms involved. The available data[19,51] (and H.G. and G.d.H., unpublished observations) are only partially consistent among different laboratories, but various studies have identified a general downregulation of lymphoid genes and a general upregulation of myeloid genes, such as runt-related transcription factor 1 (*RUNX1*), in aged HSCs[19], which is consistent with a skewing of aged HSCs towards myeloid differentiation. Molecules that are involved in cell–cell interactions, such as P-selectin and intercellular adhesion molecule 1 (ICAM1), and molecules that are involved in the nuclear factor-κB (NF-κB) response showed higher expression in aged HSCs compared with young HSCs, which indicates that aged HSCs might have a pro-inflammatory status[51].

Similarly, the expression of genes associated with protein folding, including heat shock protein 8 (*Hspa8*) and *Dnajc3 (*DNAJ (also known as HSP40) homolog subfamily C3), was found to be upregulated in aged HSCs[51], which indicates that protein integrity might be compromised with ageing. Indeed, increased protein damage and protein misfolding are observed in aged fibroblasts, so similar events might take place in aged HSCs[52]. Thus, in general, the differences in the expression profiles of young versus aged HSCs not only provide an opportunity to define novel molecular biomarkers of stem cell ageing (for example, P-selectin) but they might also indicate possible mechanisms of ageing in HSCs and other stem cells.

mtDNA mutations, metabolism and oxidative damage. The mitochondrial ageing theory states that oxidative damage, which is mediated primarily through the production of reactive oxygen species (ROS), affects the replication and the transcription of mitochondrial DNA (mtDNA) and results in a decline in mitochondrial function. This in turn creates a feedback loop, in which ROS production is increased and further promotes damage to mtDNA. This cumulative damage in mitochondria is thought to contribute, along with telomere attrition (see below), to replicative senescence and cellular ageing, particularly in differentiated cells like fibroblasts.

Indeed, increased levels of mtDNA mutations, including point mutations and deletions, as well as higher levels of ROS, are found in aged tissue such as the aged skin and muscles[53]. Moreover, transgenic mice that have increased mtDNA mutations as a result of a lack of the mitochondrial DNA polymerase catalytic subunit gamma (POLG) expression develop lymphopenia and anaemia, which indicates that mtDNA damage, ROS and haematopoiesis are linked[54]. The fact that the reduced self-renewal potential of HSCs in serial transplantation experiments can be rescued by *N*-acetyl cysteine (NAC)-mediated ROS inhibition[55] further implies a role for ROS in HSC ageing. Remarkably, HSCs that have high intracellular levels of ROS also show increased activation of the p38 mitogen-activated protein kinase (MAPK) and mammalian target of rapamycin (mTOR), both of which have been shown to induce exhaustion of stem cells after serial transplantation[56]. In this context, it is also interesting that HSCs from mice lacking the FOXO protein family of transcription factors — which, like mTOR, are an integral part of the complex of the insulin–insulin growth factor 1 (IGF1) signalling network and are therefore involved in the regulation of cell metabolism — exhibit higher levels of ROS than wild-type mice, and this is accompanied by short-term hyperproliferation and increased HSC apoptosis[57,58]. Interestingly, forkhead box O3A (FOXO3A) is one of the few confirmed human longevity genes[59].

Although these data demonstrate a role for mitochondrial mutations, ROS and the control of cell metabolism in the self-renewal of HSCs and therefore, implicitly, in HSC ageing, novel data comparing the levels of ROS in physiologically aged HSCs and in HSCs from *Polg*$^{-/-}$ mice indicate that ROS levels are actually not elevated in physiologically aged HSCs. In addition, these studies show that although intact mitochondrial function is required for appropriate multilineage stem cell differentiation, mtDNA mutations do not directly affect HSC function. These data call into question the hypothesis that ROS and mtDNA mutations directly affect HSC ageing[60], which reinforces the need for additional research to unequivocally determine the role of mtDNA mutations, metabolic stress and oxidative damage in HSC ageing.

DNA damage and telomere shortening. In another prominent theory to explain the general mechanisms of cellular ageing, the accumulation of genomic DNA damage and telomere erosion — a particular form of DNA damage — have been implicated as important underlying causes of this process, as has been shown to occur in fibroblasts. Such postulated mechanisms of ageing fit into the current ageing hypotheses, which (in their most simplified form) suggest that damaged macromolecules accumulate with age and interfere with cellular functions, thereby resulting in senescence. Telomeres are repetitive DNA sequences at the ends of chromosomes that prevent the activation of the DNA damage response and DNA repair activity. In the absence of telomerase, the length of the telomeres is reduced after each round of DNA replication as well as in response to increased levels of ROS. Telomere shortening gives rise to unprotected ends of chromosomes that are then recognized as DNA damage, as has been shown to occur in embryonic stem cells[61]. Thus, the decreased regenerative capacity of aged HSCs might result from an age-dependent accumulation of DNA damage[1,62,63] or it might be linked to telomere shortening[64,65].

Quiescence
A non-cycling resting state in the G0 phase of the cell cycle that is important for long-term function.

Reactive oxygen species
(ROS). Highly reactive molecules that consist of several diverse chemical species including the superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2). Because of their potential to cause oxidative deterioration of DNA, proteins and lipids, ROS have been implicated as one of the causative factors of ageing. As ROS are generated mainly as by-products of mitochondrial respiration, mitochondria are thought to be the primary target of oxidative damage.

Replicative senescence
An inability of most cell types to divide indefinitely owing to terminal cell cycle arrest after a defined number of cell cycles, probably as a result of telomere attrition.

Mammalian target of rapamycin
(mTOR). A conserved serine/threonine kinase regulating metabolism and the expression of growth factors in response to environmental cues. mTOR activity is inhibited by the drug rapamycin.

FOXO protein family
A subgroup of the forkhead box (FOX) family of transcription factors. FOXO proteins are regulated by the insulin–phosphoinositide 3-kinase–AKT signalling pathway.

Telomere
A repetitive nucleotide sequence at the end of chromosomes that protects the ends from deterioration or from fusion with neighbouring chromosomes.

Telomerase
An enzyme that is capable of extending the ends of telomeres after replication using an RNA template that is part of the enzyme complex. This counteracts telomere shortening after each cell division cycle.

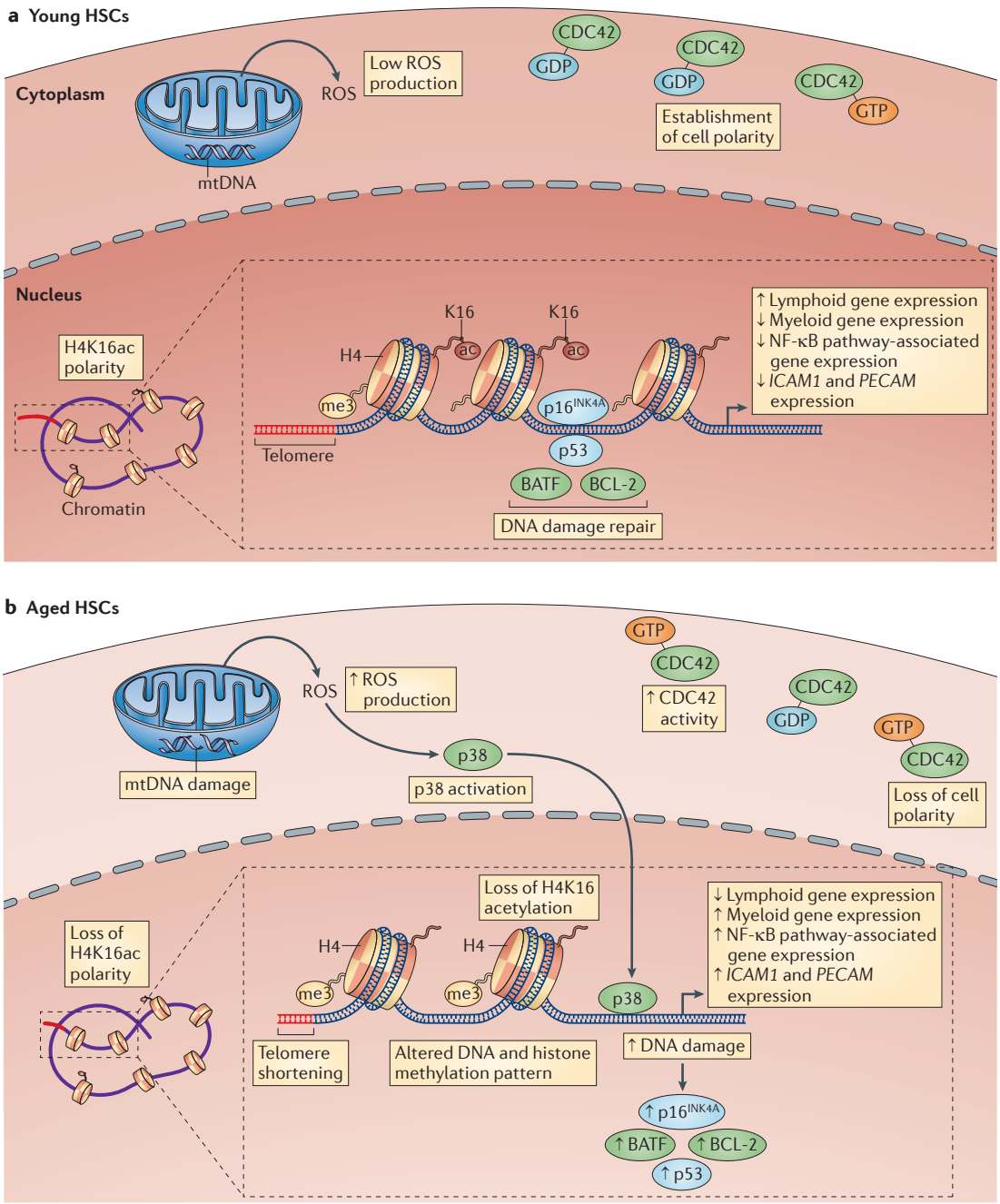


Figure 2 | **Cell-intrinsic mechanisms of HSC ageing. a** | Two of the hallmarks of young haematopoietic stem cells (HSCs) are the low production of reactive oxygen species (ROS) by mitochondria and the establishment of cell polarity by the cell division control protein 42 (CDC42). CDC42 establishes cell polarity through both its localization and its activity status (as determined by the ratio of GTP-bound CDC42 to GDP-bound CDC42). In addition, in young HSCs DNA integrity is achieved through the maintenance of telomere length and through the activation of effective DNA damage repair responses. Also, specific chromatin modifications, including acetylation of lysine 16 on the tail of histone H4 (H4K16ac) and maintenance of high expression of lymphoid genes, are involved. **b** | In aged HSCs, increased CDC42 activity causes depolarization of planar cell polarity markers in the cytoplasm, as well as loss of epigenetic polarity for H4K16ac in the nucleus. The mitochondrial production of ROS is increased, leading to mitochondrial DNA damage and increased activation of p38 mitogen-activated protein kinase, which might be involved in HSC ageing. Moreover, the altered gene expression profiles in aged HSCs indicate a mechanistic role for nuclear factor-κB (NF-κB) and stress adhesion signalling in ageing. Increased DNA damage and telomere shortening in aged HSCs induce the expression of cyclin-dependent kinase inhibitor 2A (CDKN2A; also known as $p16^{INK4A}$) and probably of B cell lymphoma 2 (BCL-2), B cell-activating transcription factor (BATF) and p53. The resulting DNA damage signalling might cause senescence, apoptosis or differentiation. Changes in the chromatin epigenetic state, including DNA or histone methylation, or histone acetylation (such as H4K16ac) indicate improper replication of epigenetic marks upon cell division or reduced genome-wide levels of acetylation upon ageing. *ICAM1*, intercellular cell adhesion molecule 1; mtDNA, mitochondrial DNA; PECAM, platelet/endothelial cell adhesion molecule.

In support of this theory, both human and mouse aged HSCs have increased levels of DNA damage, as determined by H2AX foci formation in the nucleus, compared with young HSCs[63,66]. Moreover, the third generation of mice with dysfunctional telomerase, which have critically shortened telomeres, show premature ageing of HSCs as well as other types of stem cells[67]. However, it is still unclear whether telomeres shorten sufficiently in stem cells during the lifetime of mice and humans to result in unprotected chromosome ends[68,69]. In addition, mouse HSCs express low levels of telomerase, and the overexpression of telomerase in HSCs does not improve their self-renewal capacity in serial transplantation assays[70]. It is worth noting that only a small number of genes that are involved in DNA repair and in the stress response, such as X-ray repair cross-complementing protein 1 (*Xrcc1*), Bloom syndrome protein (*Blm*) and XPA binding protein 2 (*Xab2*), are differentially expressed in young and aged HSCs[51]. Furthermore, mice deficient in crucial DNA repair genes, such as xeroderma pigmentosum group D complementing protein (*Xpd*; also known as *Ercc2*), Ku autoantigen 80 kDa subunit (*Ku80*; also known as *Xrcc5*) and mRNA transport regulator 3 (*Mtr3*; also known as *Exosc6*) (the RNA component that is necessary for telomerase function), do not show a depletion of HSCs upon ageing under steady-state conditions[62]. These findings reinforce the need for additional research to define the role of telomeres as well as DNA damage and repair in HSC ageing.

DNA damage triggers signalling cascades that lead to cell cycle checkpoint activation, apoptosis or differentiation. Several important proteins are involved in these pathways, including the tumour suppressor protein p53, cyclin-dependent kinase inhibitor 2A (CDKN2A; also known as $p16^{INK4A}$), the anti-apoptotic members of the BCL-2 family and the recently identified B cell-activating transcription factor (BATF), which promotes the lymphoid differentiation of HSCs in response to gamma-irradiation[71,72]. The potential role of p53 in HSC ageing is still controversial[73], and the roles of BCL-2 and BATF have not yet been reported[71,74]. Increased expression of $p16^{INK4A}$ is seen in many aged cells, including in aged HSCs[75], and the absence of $p16^{INK4A}$ in HSCs seems to ameliorate at least some of the phenotypes that are associated with their ageing, namely the increase in their number and their compromised self-renewal capacity in serial transplantation assays. However, the reported effect of $p16^{INK4A}$ on HSC ageing in transplantation experiments is modest, and in other studies no role was found for $p16^{INK4A}$ in the steady-state ageing of HSCs[76]. These findings indicate that $p16^{INK4A}$ might contribute to HSC ageing under certain, but not all, circumstances, although the mechanisms involved are still unclear[75].

Current data support a role for the accumulation of DNA damage in the ageing of fibroblasts and other differentiated cells[77]. However, DNA damage and the resulting mutations alone seem unlikely to have a direct role in driving the ageing of HSCs. By contrast, there is accumulating evidence that the signalling pathways that are induced downstream of the DNA damage response are involved in the ageing of HSCs[78]. Thus, the regulation and the function of such signalling pathways might be more important for HSC ageing than DNA damage itself.

Epigenetic signature of ageing. The self-renewal capacity of stem cells relies on the accurate transmission of epigenetic information to daughter cells. The relevance of epigenetic stability in stem cell ageing is only now gradually emerging. The epigenetic status of DNA or histones (BOX 2) is dynamically regulated by large multisubunit protein complexes that recruit chromatin-modifying enzymes via as yet undefined sequence-specific DNA binding.

At the single-cell level, HSCs that showed a balanced differentiation potential when they were young have been shown to give rise to myeloid-biased stem cells upon ageing[28]. This indicates that the epigenetic programmes that maintain HSC function deteriorate with age; indeed, recent publications have reported changes in DNA methylation patterns in primitive haematopoietic cells upon ageing[79–81]. The failure of DNA methyltransferases (DNMTs), histone methyltransferases or histone acetyltransferases to copy HSC-specific DNA and histone modifications to newly formed DNA strands might underlie the altered epigenetic status of aged HSCs[82]. In addition, HSC-specific epigenetic marks might be asymmetrically inherited by daughter cells as a consequence of the non-random segregation of template DNA strands[83].

Furthermore, genes that are involved in chromatin remodelling and in chromatin-dependent transcriptional silencing are downregulated in aged HSCs; these include genes related to the chromatin remodelling SWI/SNF complex, such as *Smarca4* (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 4), *Smarcb1*, histone deacetylase 1 (*Hdac1*), *Hdac2* and *Hdac6*. Genes that are involved in the control of DNA accessibility — such as sirtuin 2 (*Sirt2*), *Sirt3* and *Sirt7* — are also downregulated in aged HSCs[51]. There has been much interest in epigenetic modifier genes, as the idea that ageing is predominantly driven by changes in the genome-wide epigenetic signature of HSCs has been supported by the recent observation that the level of acetylation of histone H4 on lysine 16 (H4K16) is actually reduced in HSCs upon ageing and that it might have an important causative role in this process[25].

It is now evident that members of the mammalian Polycomb repressive complexes (PRCs) have an essential role in ensuring that proper replication of histone modifications occurs during blood cell development. The combined activities PRC1 and PRC2 involve chromatin compaction and gene repression. The canonical model of Polycomb-mediated gene repression includes the deposition of trimethylated H3K27 (H3K27me3) marks by the histone methyltransferase enhancer of zeste homolog 2 (EZH2), after which H3K27me3 is recognized by the chromobox protein

H2AX
Histone 2A family, member X; phosphorylated H2AX is a sensitive marker for DNA double-strand breaks. Almost every DNA double-strand break forms a H2AX focus, but whether every H2AX focus identifies a double-strand break remains controversial.

p53
A tumour suppressor protein that responds to diverse cellular stresses by regulating target genes that induce cell cycle arrest, apoptosis, senescence, DNA repair or changes in metabolism.

$p16^{INK4A}$
A cyclin-dependent kinase inhibitor that stabilizes cell cycle arrest by activating the checkpoint activity of the retinoblastoma protein.

BCL-2 family
A family of proteins containing at least one BCL-2 homology (BH) region. The family is divided into anti-apoptotic multidomain proteins (such as BCL-2 and BCL-XL), which contain four BH domains (BH1, BH2, BH3 and BH4), pro-apoptotic multidomain proteins (for example, BAX and BAK), which contain BH1, BH2 and BH3 domains, and the pro-apoptotic BH3-only family (such as BH3-interacting-domain death agonist (BID), BCL-2-interacting mediator of cell death (BIM) and p53-upregulated modulator of apoptosis (PUMA)).

Epigenetic information
Non-genetic information that promotes alternative cell states and is regulated by developmental and environmental cues. Epigenetic information maintains distinct phenotypes among cells that share identical DNA sequences.

DNA methyltransferases
(DNMTs). Enzymes that transfer methyl groups from *S*-adenosylmethionine to specific adenines or cytosines in DNA.

Box 2 | **Epigenetics and HSCs**

Regulation of stem cell activity through epigenetic modifications might involve changes to the DNA methylation, histone methylation and histone acetylation status. DNA methylation involves the covalent attachment of a methyl group to the C5 position of cytosine residues in CpG dinucleotide sequences, and this modification is frequently referred to as mCpG. CpG methylation generally results in gene repression[141]. Recent findings suggest that cytosine residues other than those in CpG can also be methylated in stem cells and that the methylation of these non-CpG cytosines is crucial for gene regulation in embryonic stem cells, whereas induced pluripotent stem cells show aberrant reprogramming of CpG methylation[142,143]. It is not yet known whether the genome-wide CpG methylome is different between young and aged haematopoietic stem cells (HSCs).

Histones can be modified by methylation and acetylation, as well as by phosphorylation and ubiquitylation[93,144,145]. Histone modifications are generally considered to be more reversible than DNA methylation. Both histone methylation and histone acetylation are so far the best characterized modifications with respect to regulation of the chromatin status and DNA transcription. Histones can be methylated or acetylated at lysine residues. The histone methylation status can comprise monomethyl, dimethyl or trimethyl marks, and is regulated by DNA methyltransferases (DNMTs) and histone lysine demethylases. Histone methylation marks that are commonly associated with gene activation are H3K4me2 (dimethylated histone H3 on lysine 4), H3K4me3, H3K79me2 and H3K79me3. Histone methylation marks that are commonly associated with gene silencing are H3K9me1, H3K9me2, H3K9me3, H3K27me1, H3K27me3, H4K20me2 and H4K20me3.

The histone acetylation status is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), and usually involves the addition of just one acetyl moiety to a lysine. Histone acetylation is frequently associated with gene activation. H3K9ac (acetylation of lysine 9 on histone 4), H3K56ac, H4K5ac, H4K12ac and H4K16ac are the most common histone acetylation marks.

Some of the above mentioned epigenetic marks have been reported to alter with age in differentiated tissues, but it remains inconclusive whether ageing results in a more repressed or a more activated chromatin status[146]. With the exception of H4K16ac, which is decreased with ageing, no other changes have yet been reported for the histone methylation status and acetylation status in aged HSCs. However, HSCs that lack histone-modifying enzymes such as the sirtuin (SIRT) deacetylases have an ageing-associated phenotype, and gene expression studies have documented a reduction in the expression of several epigenetic modifiers with age[51]. These findings indicate that epigenetic regulation might be involved in HSC ageing.

Finally, in addition to DNA and histone modifications, cellular polarity (the distribution pattern of proteins, RNA or DNA within a cell) might also regulate access to genetic information and thus might qualify as an epigenetic mark (referred to as 'epigenetic polarity' or 'epi-polarity')[25,147]. Recent evidence has implicated cell polarity in HSC ageing.

Histone methyltransferases
Enzymes that catalyse the transfer of methyl groups to lysine and/or arginine residues on histones; the most well-studied histone methyltransferase is SUV39H1 (suppressor of variegation B9 homolog 1) and its mammalian homologues, which methylate histone H3 on lysine 9.

SWI/SNF complex
An ATP-dependent chromatin-remodelling protein complex that was initially identified in yeast. Related complexes exist in mammals and are involved in the remodelling of chromatin in various genes.

Polycomb repressive complexes
(PRCs). Groups of proteins that maintain gene expression states throughout development by regulating chromatin structure. In mammals there are two core Polycomb complexes: PRC1 and PRC2. PRC1 catalyses the monoubiquitylation of histone H2A. Both complexes contribute to chromatin compaction. PRC2 harbours the histone methyltransferases EZH1 (enhancer of zeste homolog 1) and EZH2, which catalyse the methylation of histone H3 at lysine 27. These two complexes are involved in differentiation, in the maintenance of cell identity and proliferation, and in stem cell plasticity.

H3K4 trimethylation complex
A complex of proteins that are involved in the addition of three methyl groups to histone H3 on lysine 4.

homologue (CBX) in the BMI1-containing PRC1. Aberrant expression of multiple members of PRC1 or PRC2, including BMI1, RING1, MEL18 (also known as PCGF2), embryonic ectoderm development (EED) and EZH2, have been shown to dysregulate blood cell formation in mice[84]. One of the key target loci of PRC1 is the *Ink4a–Arf* (also known as *Cdkn2a*) locus, which encodes $p16^{INK4A}$. It has been demonstrated that stem cell defects that result from the loss of BMI1 can be partially restored by the deletion of *Ink4a–Arf*[85]. However, the function of $p16^{INK4A}$ in the normal ageing of HSCs remains a matter of debate[76], and it is probable that several other PRC1 target loci exist.

As gene expression studies have reported a reduction in the expression of several epigenetic modifiers in aged murine HSCs, other epigenetic mechanisms might also contribute to ageing[51]. Although the role of the Polycomb group genes and the epigenetic modifications that these proteins induce are probably the best understood, additional epigenetic modifications have been associated with ageing phenotypes in other models. For example, the H3K4 trimethylation complex seems to regulate lifespan in a germline-dependent manner in *Caenorhabditis elegans*[86]. Also, somatic mutations have been reported in the methylcytosine dioxygenase TET2 in otherwise healthy elderly individuals who have clonal haematopoiesis[87]. TET2 catalyses the hydroxylation of 5-methylcytosine throughout the genome, which results in a global loss of DNA methylation. Indeed, it has been demonstrated that normal haematopoiesis is dependent on the maintenance of accurate DNA methylation patterns[88,89]. Furthermore, it has been hypothesized that the skewing of HSC differentiation is mainly dictated by epigenetic alterations that have not yet been identified[1,51]; however, it is also possible that the altered differentiation bias of aged HSCs is caused by a gradual decline in the function of HSCs owing to the accumulation of DNA damage, or other cellular damage, with time[62,90,91].

Although the transcriptome of young and aged mouse HSCs has been assessed in several microarray studies[19,51,92], genome-wide analyses of epigenetic modifications in young and aged HSCs have not yet been carried out, with the exception of screening for H4K16 acetylation (see above) and DNA methylation[79]. Therefore, at present there is no definitive proof that causally links the erosion of epigenetic DNA or histone marks with the impaired function of aged HSCs. Such studies are predominantly hampered by technical and methodological issues, which preclude quantitative measurements and functional assays from being carried out on very small numbers of cells. Importantly, if epigenetic modifications have a key role in HSC ageing, epigenetic regulation could be used therapeutically to reverse the epigenetic signature of aged stem cells; pharmacological compounds that alter epigenetic signatures, such as decitabine and valproic acid, are already in clinical use[25,82,93].

Loss of cell polarity and the role of CDC42. Changes in cell adhesion, as has been described for aged HSCs, are frequently linked to changes in cell polarity. Indeed, recent data from different stem cell systems suggest that there is a correlation between altered cell polarity and stem cell ageing. In *Drosophila melanogaster,* for example, aged germline stem cells (GLSCs) exhibit misoriented centrosomes and thus altered cell polarity compared with young GLSCs. This correlates with the reduced self-renewal of aged GLSCs, probably as a result of the alteration of the mode of stem cell division[94].

Genome-wide association studies have shown that increased expression of the small RHO GTPase cell division control protein 42 (CDC42) in mononuclear haematopoietic cells is strongly associated with morbidity and ageing in humans[95]. CDC42 is involved in regulating the three-dimensional distribution of proteins and organelles[96]. CDC42 functions as a binary molecular switch that is turned on in the GTP-bound state and turned off in the GDP-bound state. This occurs in response to various HSC stimuli, including growth factors, cytokines and cell–cell or integrin-mediated cell–extracellular matrix (ECM) interactions. Novel data demonstrate that CDC42 activity is substantially increased in aged HSCs and that this contributes to HSC ageing[25]. The increased activity of CDC42 results in a loss of cell polarity in aged HSCs, which involves the random distribution of tubulin, CDC42 itself and other planar cell polarity markers, as well as random nuclear distribution of acetylated H4K16 (epigenetic polarity; see below)[25]. Therefore, HSC ageing seems to be intricately related to a loss of cell polarity, as has already been described for other stem cell systems[97,98].

In summary, stem cell-intrinsic mechanisms strongly contribute to HSC ageing. Although the disruption of mitochondrial function, the accumulation of DNA damage and the erosion of telomeres are frequently seen as mechanisms of cellular senescence in differentiated cells, recent data do not support a direct role for DNA damage in HSC ageing, but instead indicate that downstream signalling is involved in the defective function of aged HSCs. In addition, new research approaches have highlighted a central role for epigenetic mechanisms, including changes in cell polarity and the distribution of epigenetic markers, in HSC ageing.

Cell-extrinsic mechanisms involved in HSC ageing

As discussed above, the molecular mechanisms that are involved in HSC ageing are thought to be primarily intrinsic to the stem cell compartment. However, some new and exciting data also highlight a potentially important role for HSC-extrinsic factors in driving or exacerbating some aspects of HSC ageing (FIG. 3). In this section, we summarize and interpret recently published data showing the impact of niche-secreted and systemic factors on HSC ageing.

Niche-derived factors that influence HSC ageing. The bone marrow niche (BOX 1) interacts dynamically with HSCs to coordinate their survival, proliferation, self-renewal and differentiation[99–103]. HSC niches are composed of several different cell types, and it has been suggested that HSC fates are regulated by a multitude of locally secreted factors, including — among others — activators of Notch signalling, WNT ligands, angiopoietins, stem cell factor (SCF; also known as KITLG) and thrombopoietin (TPO)[46]. Although recent publications have started to elucidate the mechanisms through which niches regulate HSCs, very few data are available on the differences between young and aged HSC niches. Changes in niche composition or function upon ageing involve decreased bone formation, enhanced adipogenesis and changes in ECM components[29,104,105].

To measure the effects of niche-secreted factors on HSC ageing and especially to investigate the molecular mechanisms through which bone marrow stromal cells might affect HSC ageing, aged and young bone marrow cells have been transplanted into aged and young mice in a competitive setting. In contrast to what has been seen almost as a dogma in HSC biology, recent studies have shown that the aged HSC microenvironment contributes, at least partially, to the skewing of aged HSCs towards a myeloid lineage, through the secretion of increased levels of the pro-inflammatory CC-chemokine ligand 5 (CCL5; also known as RANTES)[42]. This finding shows that an aged niche can influence not only the regenerative capacity but also the differentiation potential of aged HSCs. There is also evidence to suggest that the aged microenvironment promotes the expansion of the number of pre-leukaemic HSCs in the context of myeloproliferative disorders[43,106].

CXC-chemokine ligand 12 (CXCL12; also known as SDF1) is an important chemokine produced by osteoblasts in the bone marrow niche; it functions as a chemoattractant for HSCs, regulating their localization, their turnover and their mobilization from the bone marrow. A recent study showed that the increased fat content in the bone marrow of elderly individuals (which is attributed to the preferential differentiation of bone marrow mesenchymal stem cells into adipocytes) negatively correlates with plasma levels of CXCL12 (which are decreased in elderly individuals) and positively correlates with HSC numbers in the bone marrow (which are increased in elderly individuals)[107]. An increase in ROS production and a decrease in the hypoxic conditions of the osteoblastic niche through as yet unknown mechanisms are also among the early age-associated changes in the niche[29,108], which further indicates that the aged microenvironment might have a negative influence on the maintenance of HSC function.

Interestingly, connexin 43 (CXN43; also known as GJA1) forms gap junction channels between HSCs and stromal cells, and these gap junction channels maintain low ROS levels in HSCs and might thereby preserve HSC function[109,110]. Indeed, during genotoxic stress a substantial fraction of newly generated ROS are eliminated through the CXN43 gap junction channels between HSCs and stromal cells, and they are deposited in niche cells rather than being confined to HSCs[111]. This avoids activation of p38 MAPK and the downstream $p16^{INK4A}$, and therefore prevents HSC senescence in response to

Cell polarity
The asymmetric distribution of proteins, lipids and/or their complexes within the cell. It is believed that polarity determines the mode of cell division and thus the fate of the two daughter cells.

Centrosomes
Cytoplasmic organelles that organize the microtubules. Preceding mitosis, the centrosome doubles and is then involved in the generation of the mitotic spindle for subsequent chromosome segregation during mitosis. In many cell types the centrosome is directly located in the centre of the cell and is therefore assigned a polar distribution, in which case cells frequently undergo asymmetric divisions.

Mode of stem cell division
An symmetric or asymmetric event, with respect to the potential of the daughter cells. An asymmetric division balances stem cell self-renewal and differentiation through the production of one stem cell and one differentiating cell, whereas a symmetric division might result in either two stem cells or two differentiated cells.

Osteoblasts
Cells of mesenchymal origin that are responsible for the formation of bone. Osteoblasts are thought to be a crucial component of the haematopoietic stem cell niche.

Mesenchymal stem cells
(MSCs). Multipotent progenitor cells originally identified in the bone marrow stroma. MSCs can be expanded *in vitro* and, under appropriate conditions, give rise to several cell types, including bone and fat precursors.

Gap junction channels
Channels composed of integral membrane proteins called connexins. Gap junction channels connect the cytoplasms of adjacent cells, allowing for the diffusion of ions and small metabolites between cells.

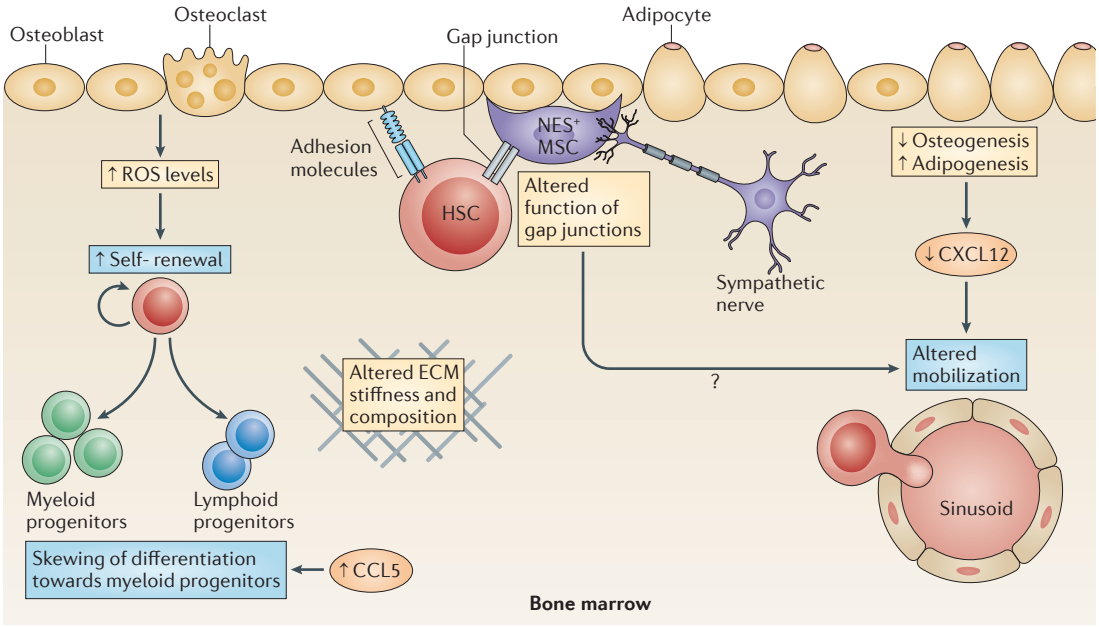


Figure 3 | **The contribution of the niche to HSC ageing.** The haematopoietic stem cell (HSC) niche undergoes several changes with ageing. The number of osteoblasts decreases, which leads to reduced osteogenesis. Moreover, osteoblasts generate higher levels of reactive oxygen species (ROS), the number of adipocytes increases as a result of the skewed differentiation of aged mesenchymal stem cells (MSCs) and the composition of the extracellular matrix (ECM) is altered. Increased ROS levels induce p38 mitogen-activated protein kinase (MAPK) signalling in HSCs (not shown), which is associated with a reduced self-renewal capacity. Increased adiposity and reduced osteogenesis might be connected to decreased CXC-chemokine 12 (CXCL12; also known as SDF1) levels in the niche upon ageing. However, it is still unclear how these changes in the number of niche cells affect HSC ageing. Extrinsic factors that are secreted by cells that form the HSC niche promote the skewed differentiation of aged HSCs towards myeloid progenitors, and inhibit the development of lymphoid progenitors. This might partially be the consequence of increased levels of several cytokines and chemokines, such as CC-chemokine ligand 5 (CCL5; also known as RANTES), in the niche. Dysregulated cytokine and chemokine expression, as in the case of CXCL12, in the bone marrow niche might also contribute to the altered mobilization of aged HSCs. The sympathetic nervous system, which controls the HSC niche by regulating the interactions of HSCs with nestin-positive (NES$^+$) MSCs, might also be important for the regulation of HSC mobilization and might show an altered activation status upon ageing, although this has yet to be determined. It is also a possibility that the number of NES$^+$ MSCs changes with age. In addition, changes in adhesion receptor-mediated interactions in the aged niche, as well as altered cell–cell communication as a result of the altered function of gap junction channels, might cause an aged phenotype in HSCs.

ROS-mediated stress[111]. As mentioned above, both p38 MAPK and p16^{INK4A} have been implicated in segmental ageing-associated HSC phenotypes, so it is tempting to speculate that such a novel mode of stromal cell–HSC interaction might also regulate aspects of HSC ageing under conditions of stress.

In summary, these findings emphasize that there might be crosstalk between the aged HSC niche and HSC-intrinsic mechanisms to promote HSC ageing. Therefore, it can be predicted that the list of niche factors that influence stem cell ageing might increase in the next couple of years to include as yet unidentified cytokines and other signalling molecules, including adhesion molecules[112].

Systemic factors that influence HSC ageing. Several systemic factors that are secreted at distal sites and circulated throughout the body, including regulators of WNT signalling[113], cytokines, hormones and neuropeptides[114], have been previously reported to contribute to the ageing of muscle stem cells. It is also known that systemic factors can affect the bone marrow niche microenvironment[115,116]. Although systemic factors potentially contribute to the age-dependent dysfunction of HSCs, the nature and identity of these factors remains largely unknown.

An interesting group of systemic factors that can influence the HSC compartment are those that are secreted by the sympathetic nervous system. Indeed, sympathetic signals can influence bone marrow homeostasis by inducing haematopoietic stem or progenitor cell mobilization. Unidentified sympathetic signals contribute to a dramatic reduction in the function of osteoblasts following granulocyte colony-stimulating factor (G-CSF; also known as CSF3) administration[102]. Osteoblasts have been reported to express receptors for several neuropeptides, which indicates that these cells could integrate several neuronal signals[117]. Given that the levels of neuropeptides might be altered with ageing[113], they might influence the self-renewal and turnover of

HSCs in the aged bone marrow niche. It is worth noting that several other systemic factors might influence HSC ageing indirectly by regulating the levels of circulating neuropeptides. For example, it was recently shown that a systemic increase in the chemokine CCL11 (also known as eotaxin) negatively regulates neurogenesis and cognitive function[118].

Therefore, recent data reveal that both niche-derived and systemic factors have a role in HSC ageing, and these data also challenge the previous view that HSC ageing is only driven by cell-intrinsic mechanisms. Additional studies are clearly required to better understand the role of the niche, as well as systemic factors, in modulating HSC function upon ageing.

Conclusions: is HSC ageing reversible?

HSC ageing is an underlying cause of immunosenescence and can be defined by a set of cell phenotypes. HSC ageing involves several cell-intrinsic and cell-extrinsic molecular mechanisms, including epigenetic regulation and signalling by both niche-derived and systemic factors. Therefore, a clinically important question is whether HSC ageing is permanent or whether it can be reversed.

John B. Gurdon and Shinya Yamanaka were awarded the Nobel Prize in medicine in 2012 for the observation that cellular differentiation is reversible, which proved that stem cell specification and changes in cells over time are generally not associated with irreversible modifications. For it to be reversible, HSC ageing would need to be primarily driven by reversible molecular changes, such as chromatin and protein modifications, and changes in protein expression or changes in signalling, which are triggered by fluctuating levels of extrinsic factors rather than resulting from irreparable damage (such as DNA mutations). Indeed, as discussed above, this seems to be the case.

So far, only few experimental data are available showing pharmacological approaches to rejuvenate mammalian stem cell function either directly, by targeting HSCs, or indirectly, by targeting the stem cell niche. Thus the causal mechanisms of stem cell ageing still remain largely unknown[25,119,120]. However, there is compelling evidence that muscle stem cells can be rejuvenated by enhancing the activation of the Notch–Delta pathway and by restoring satellite cell proliferation[120]. In HSCs, mTOR and CDC42 are activated with ageing, and have been identified as promising pharmacological targets for HSC rejuvenation[25,119,121]. The functions of mTOR and CDC42 are evolutionarily conserved[122–124], and targeted pharmacological approaches to reduce CDC42 or mTOR activity were sufficient to reverse most of the ageing-associated HSC phenotypes in the case of CDC42, and several in the case of mTOR. Thus, current data support the idea that rejuvenation of aged HSCs could be achieved by pharmacological targeting of cell-intrinsic ageing mechanisms.

Interestingly, both CDC42 and mTOR have also been implicated in organism longevity. Increased activity of CDC42 is associated with mortality in the elderly[95] and results in a premature-ageing-like syndrome in mice[125]. Genetic attenuation of mTOR signalling increased lifespan in *Saccharomyces cerevisiae*, *C. elegans* and *D. melanogaster*, whereas inhibition of mTOR with rapamycin extended lifespan in yeast, flies and mice[126–131]. So, it is tempting to speculate that the targeting of these proteins *in vivo* might result in the rejuvenation not only of HSCs but also of the whole organism. Indeed, an appealing idea is that by rejuvenating tissue-specific stem cells it might be possible to rejuvenate tissues and, ultimately, the whole organism[82,132,133]. Genome-wide linkage analyses actually support a role for HSCs in regulating lifespan, as genetic loci that control lifespan in mice are also linked to ageing phenotypes in primitive haematopoietic cells[20,134].

Moreover, single cell-whole genome sequencing might show whether HSCs do have an increased genetic mutational load upon ageing; if this is the case, it still remains unclear as to whether this is causative of ageing. However, efforts to rejuvenate HSCs might need to take into account the pre-existing genetic mutational load as this might increase the risk of side effects such as tumorigenesis. In addition to changes in cell signalling, epigenetic changes correlate with HSC ageing. Future research will clarify whether epigenetic modifications really have a crucial role in HSC ageing. Although HSCs reportedly show changes in their DNA methylation pattern with age, other epigenetic changes have not yet been fully characterized in ageing HSCs. This will require approaches to determine the direct role of the changes, using, for example, single-cell chromatin immunoprecipitation (CHIP) tools as well as experiments to investigate the importance of the proteins that regulate these changes.

Although the observations mentioned above indicate that reversal of the cell-intrinsic parameters of ageing might be sufficient to achieve rejuvenation, HSC-extrinsic factors such as circulating cytokines and niche-specific factors should perhaps also be targeted, especially in the context of rejuvenation *in vivo*. Overall, HSC ageing seems to be driven by several interconnected mechanisms. So, future progress in the field might greatly benefit from a systems biology approach. Such an approach would possibly help to identify the key causes or drivers of HSC ageing and to further separate them from their consequences. Moreover, systems biology studies might show additional pharmacological approaches to ameliorate or to reverse HSC ageing.

Finally, most studies on HSC ageing are now being carried out in mice, and these studies often show that heterogeneity exists in ageing-associated HSC phenotypes among different mouse strains[134]. This further complicates attempts to apply HSC-targeting therapeutic approaches that were successful in mice to humans. Consequently, we hypothesize that a better molecular understanding of the epigenetic regulatory networks, of the DNA damage signalling pathways and of the HSC–niche interactions in ageing HSCs in both mice and humans will be required to improve existing strategies and to design novel, rational strategies for successful HSC rejuvenation.

CCL11
CC-chemokine ligand 11; a member of the CC-chemokine family and selectively recruits eosinophils. Increased CCL11 levels in the blood are found in aged mice and humans.

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The authors declare no competing financial interests.

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