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Hematopoietic stem cell aging

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Aging is organized in a hierarchy, in which aging of cells results in aged tissues, ultimately limiting lifespan. For organ systems that also in the adult depend on stem cells for tissue homeostasis like the hematopoietic system that forms immune cells, it is believed that aging of the stem cells strongly contributes to aging-associated dysfunction. In this review, we summarize current aspects on cellular and molecular mechanisms that are associated with aging of hematopoietic stem cells, the role of the stem cell niche for stem cell aging as well as novel and encouraging experimental approaches to attenuate aging of hematopoietic stem cells to target immunosenescence.

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Introduction

A number of theories have been proposed regarding the cellular and molecular mechanisms regulating aging, and genetic, behavioral and environmental factors may all be involved [1]. Many organs with high cell turnover (e.g., skin, intestine and blood) are composed of short-lived cells that require continuous replenishment by somatic stem cells [2–6]. Aging results in the inability of these tissues to maintain homeostasis. Stem cells were initially thought to be endowed with unlimited self-renewal capacity, and thus exempt from aging. However, there is measurable and successive age-dependent decline in stem cell activity from adulthood to old age in various organs, including intestine and muscle and the blood forming system. This age-associated decline in stem cell function leads to a decline in the regenerative capacity in humans and mice [7–13], which may limit lifespan [7,8,14–16]. Identifying the underlying mechanisms of stem cell aging may be a first step towards designing treatments for aging-associated diseases of such stem-cell based tissues.

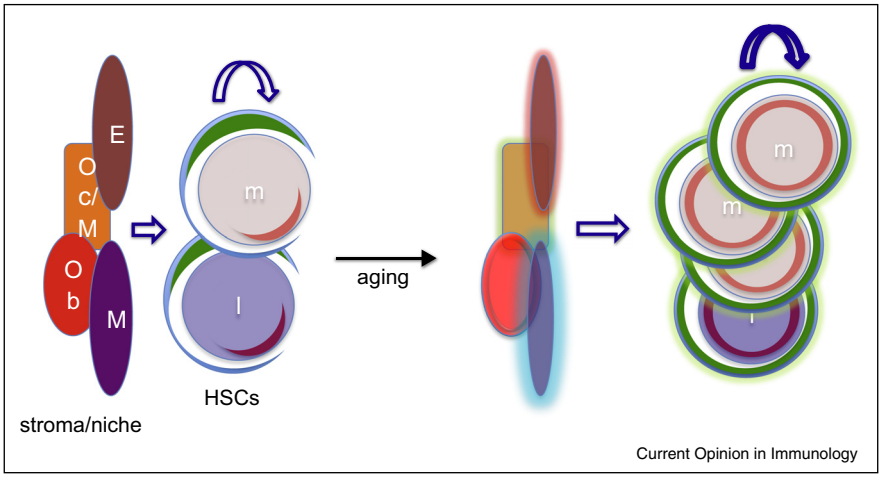
Hallmarks of hematopoietic stem cell aging — Both the innate and the adaptive part of the immune system are reduced in function upon aging. This implies as a possible underlying basic mechanism in this type of immunosenescence the dysfunction of a more primitive hematopoietic cell that contributes to both lineages, which is the hematopoietic stem cell (HSC), Figure 1.

HSCs from young and aged mice differ in their function with respect to their self-renewal and differentiation ability. HSC aging is driven by both intrinsic and extrinsic factors [8,17–21]. Referring to cell intrinsic components, we describe young HSCs and aged HSCs when speaking of HSCs from young and aged animals [22]. There are multiple aging-associated phenotypes of HSCs reported. In their sum though, and not individually, they separate young from aged HSCs. Here we shortly introduce this 'canonical' set of parameters for aging of at least murine HSCs that might serve as a general reference list.

Altered HSC self-renewal and heterogeneity

In general, aging of HSCs is associated with reduction in function. Aged HSCs show reduced self-renewal activity determined in serial transplant assays [23]. When aged HSCs are transplanted together with young HSCs into lethally irradiated young recipients, aged HSCs are on average 2-fold less efficient in contributing to hematopoiesis compared to young HSCs [24,25] and exhibit a 2-fold reduced ability to home to the bone marrow (BM) [26]. There is functional heterogeneity among primitive HSCs: some display a preferential production of myeloid offspring (and can therefore be regarded as myeloid-biased), some other show preferential production of lymphoid cells (and are referred to as lymphoid-biased), while usually HSCs with a more balanced output dominate [27–30]. An explanation for lineage skewing upon aging is therefore that the composition of the pool of HSCs changes. Such a view is supported by data that show that myeloid-biased HSCs increase at the expense of lymphoid-bias or balanced clones upon aging [31–34]. Clonal

Figure 1



Aging of HSCs has both, intrinsic elements as well is influenced by changes in stem cell niche (cell composition and cellular function). As listed in Table 1, HSCs show heterogeneity with respect to their differentiation potential (light purple: myeloid (myelo-erythroid, megakaryocytic, m) biased stem cells, dark purple lymphoid biased (l) stem cells) and it is assumed that the myeloid-biased subgroup expands upon aging, because of both stem cell intrinsic mechanisms and extrinsic (niche) mechanism. In addition, stem cells show altered differentiation upon aging, and limited self-renewal (curved arrow), which results in reduced repopulation potential. Upon aging, stem cells are located more distant from the endosteal stroma cells (straight arrow), and show more dynamic protrusions (glow), while endosteal stroma cells like osteoblasts (Ob), osteoclasts/monocytes (Oc/M), endothelial cells (E) as well as mesenchymal type of stem cells (M) that are known to be involved in forming a niche are believed to alter function upon aging (glow), contributing to impaired hematopoiesis and immune cell formation upon aging.

analyses on sorted young and aged HSCs though revealed that these myeloid-biased HSCs from aged BM also possess a reduced proliferative response and additional functional aging deficiencies, implying that all HSCs subtypes present with reduced function upon aging [35]. It has also recently reported that the frequency of adult CD41+ HSCs increased with age. CD41+ HSCs are largely quiescent and exhibit myeloerythroid and megakaryocyte gene priming, governed by Gata1, whereas CD41-HSCs were more proliferative and exhibited lymphoid gene priming, adding another aging heterogeneity marker to the HSC field [36•].

Altered HSCs differentiation

Aging also affects the differentiation potential of HSCs, which is probably the most relevant phenotype with respect to reduced immunological functions caused by changes in the hematopoietic hierarchy upon aging. Studies have demonstrated that aged HSCs are deficient in their ability to support erythropoiesis, and that aged HSCs do not efficiently generate B-lymphoid as well as T-lymphoid progeny but are better in supporting the myeloid cell lineage (see Ref. [4] and references cited), which has been linked to the skewing in HSC heterogeneity upon aging, but which is also seen on an individual stem cell level [35,37].

Table 1

Hallmarks of HSC aging

Hallmarks of HSC aging	Mouse	Human
Number/frequency	Increased [18,41••,71]	Increased [72,73]
Self-renewal	Reduced [74]	?
Heterogeneity	Altered [34,35,37], increase in CD41+ cell number (myeloerythroid and megakaryocytic primed [36•]) clonality increased [75•]	Altered [72]
Differentiation	Myeloid-biased [29,30,37]	Increased myeloid contribution [73], decreased myeloid contribution [72]
Localization	More distant from endosteum [40,41••]	?
Homing	Reduced [26]	?
Mobilization efficiency	Enhanced [38]	Normal or lower [76–78]
Stem cell polarity	Apolar [41••,70••]	?
Stem cell niche	RANTES elevated [46•] Alters clonality in HSCs upon aging [44•,45]	?

Altered HSC localization

Stem cells are supported by stroma cells and additional endogenous factors, which form the so-called stem cell niche. Aged HSCs occupy positions within BM that are distinct from position of young HSCs, and it is thus implied that they use niches that are distinct from niches young stem cells have access to. Our own data also show that aged HSCs are less efficient in their ability to adhere to stroma cells and exhibit significantly elevated cell protrusion activity *in vivo*, reducing the time for effective interactions with the microenvironment [38–40].

Altered stem cell polarity and other regulatory pathways

We recently demonstrated that the majority of aged HSC are apolar for cell polarity markers usually associated with planar cell polarity (like Scribble, Cdc42 and Dlg), while young HSCs, in their majority, show polarity for these markers [41••]. Polarity is usually associated with divisional asymmetry, while apolarity might be linked to divisional symmetry. Whether such mechanism then contributes to aging in hematopoiesis or aging-related disease in hematopoiesis like leukemia needs to be investigated in more detail. Aged HSCs exhibit distinct whole genome expression signatures [18,42] and more importantly, specific pathways that are altered upon aging can be identified, based on gene ontology terminology. We could very recently show, based initially on whole genome expression data, that aging HSC shifts from canonical to non-canonical Wnt-signaling, which is accompanied by an activation of distinct Notch signaling and calcium signaling pathways. It has been reported that aged HSC presents with increased double-strand breaks as detected by increased levels of gammaH2AX staining, a surrogate marker for DNA double strand breaks [43]. Thus, a canonical set of features phenotypically separate young from aged HSCs.

Mechanisms of HSC aging: stroma

While the list of phenotypes associated with aged HSCs is long but diverse, the underlying molecular mechanisms of stem cell aging have been more difficult to elucidate. Stem cell aging is driven not only by stem cell intrinsic factors, but also by the aging niche. For example, transplantation of young HSCs into an aged niche elicits in these young HSCs as least some of the phenotypes associated with aged HSCs, like myeloid skewing [44•,45,46•], which has been attributed molecularly to elevated expression of the factor RANTES in stroma cells in aged mice. As especially myeloid-driven leukemia increase exponentially with aging, there has also been strong interest in the community on whether aged stroma/niche can contribute to leukemia initiation or progression. More recent research clearly demonstrates [47••] that signals from the niche can actually initiate leukemia development, while research from us and others demonstrated that aged niches support the development of clonality among pre-leukemic clones (a pre-requisite for leukemia development). Together, these more recent data imply a strong role for the niche also in blood-disease development with age.

Intrinsic mechanisms of stem cell aging: SIRTs, WNTs, Notch and RhoGTPases

Current data though still support a primarily intrinsic mechanism of stem cell aging, aka the molecular mechanisms that aged stem cells are initiated in stem cells themselves and act more independently from the niche. While it has been initially difficult to identify the underlying mechanisms based on whole genome expression data, novel rational approaches, moving from aging-associated phenotypes to likely mechanistic regulators have revealed novel and exciting knowledge on underlying pathways that result in aging of HSCs.

A recent publication for example revealed that SIRT3, a mammalian sirtuin that regulates the global acetylation landscape of mitochondrial proteins and reduces oxidative stress, is highly enriched in hematopoietic stem cells (HSCs) was essential under stress or at old age, but dispensible when young [48••]. Most interestingly though, the authors showed that SIRT3 is suppressed with aging, while SIRT3 upregulation in aged HSCs improved regenerative (aka serial transplantation) capacity. While the role of sirtuins in aging has been controversially discussed, these data strongly support an important role for an acetylation regulator in the process of stem cell aging.

We recently established that the activity of the small RhoGTPase Cdc42, which cycles between an inactive, GDP bound form and an active, GTP bound forms and which functions as a molecular switch to regulate HSC polarity, differentiation and engraftment, is increased in bone marrow and other tissues upon aging. This elevated Cdc42 activity was causally linked to HSC aging [41••].

Many Wnt family proteins are expressed in hematopoietic tissues, both in stroma and in hematopoietic cells [49]. However, it remains unclear whether they function as growth or differentiation factors for HSCs. Actions of the canonical Wnt ligands are frequently opposed by non-canonical Wnts, resulting in a daunting complexity of interactions. In particular, one non-canonical signal can influence actin-dependent cytoskeletal reorganization and regulate cell polarity [50–52]. Canonical Wnt3a and non-canonical Wnt5a have been the most studied Wnt ligands in the hematopoietic context. Recently it was also shown that Wnt5a maintains long-term HSC function in the bone marrow niche, and Wnt5a signaling can affect planar cell polarity [52]. Interestingly, the elevated activity of Cdc42 in aged HSCs is a consequence of increased stem cell intrinsic expression of Wnt5a, resulting in a stem cell intrinsic shift from canonical to

non-canonical Wnt signaling as HSCs age. This aging-associated elevation of Wnt5a and Cdc42 activity in HSCs results in a loss of HSC polarity, including the acetylated form of histone 4 on lysine 16 within the nucleus, reduced engraftment, and the aging-associated differentiation skewing. In this context it is also interesting that a loss of Sirt1 in HSCs results in reduced H4K16 acetylation and transient expansion with the ultimate fate of stem cell failure [53•].

Both pharmacological inhibition of elevated Cdc42 activity and genetic inhibition of Wnt5a expression in chronologically aged HSCs rejuvenated HSC function, thus implying an important role to the Wnt5-Cdc42 pathway for aging as well as rejuvenation of aged HSCs. Interestingly, this shift in stem cell intrinsic Wnt-signaling in HSCs upon aging directly resulted in changes in Notch as well as calcium signaling upon aging of HSCs, but these changes could also be induced by exogenous Wnt5a, supporting again both intrinsic and extrinsic regulation of stem cell aging. Notch signaling in HSC aging was already implied by analyzing gene expression profiles of HSCs from patients with Down syndrome (DS) [54], as DS is associated with many of the signs of premature aging including T-cell deficiency, increased incidence of early Alzheimer-type and myelodysplastic-type diseases and leukemia. Notch-signaling of course plays a very crucial role in almost all aspects of immunity [55], so it is likely that the changes in Notch-signaling in aged HSCs influence immunosenescence.

Implications for health in aging

So what are the implications of aging of HSCs on health in aging? Conclusions on direct causal relationships between aging of stem cells and consequences for health and disease are still difficult to draw. This might also be owing to the fact that research in primarily performed in mice, and standard disease of aging in mice, except for leukemia, are very difficult to model and monitor in this model system.

Unexplained anemia is observed in older adults that cannot be accounted for by iron deficiency, renal insufficiency or other molecular/dietary problems [56]. In addition, in general, levels of red blood cells in blood tend to be lower in older adults, which are linked to frailty or other symptoms related to low oxygen levels in the system. As a consequence, older adults can show an increased susceptibility to situations of stress to the system such as infections, or additional blood loss because of surgery. Whether this unexplained anemia in older adults though is directly associated with aging of HSCs is still to be investigated in more detail in the mouse model.

The incidence of leukemia, like almost all types of cancers, increases exponentially with age. While certainly both stem cell intrinsic and extrinsic (like the niche, see above) aging mechanisms are believed to contribute to the aging-associated increase in stem cell driven leukemia like AML, the molecular contribution of aging to the elevated incidence is far from being understood, and research in this directions is still in its just beginning [44•,45,57–61].

An age-related decline in immune responses also results in greater susceptibility to infection and reduced responses to different vaccination strategies such as influenza or pneumococcal bacteria [62]. A direct influence of aging of HSCs on immune-senescence has been so far difficult to determine, as of course multiple additional parameters (e.g. thymic involution in adulthood) that are independent of stem cell biology also contribute to it [63,64]. In addition, because of the nature of HSCs, investigations need to be done in animals, precluding conclusions with respect to causal relationships. Novel interventions exist that result in long-term attenuation of stem cell aging (see above), so novel mechanistic understanding on the role of aging of HSCs on immune-senescence might be derived from analyzing immune-competence in animals with such rejuvenated hematopoiesis.

Future directions of research: attenuation of stem cell aging

It has been reported that aged muscle stem ('satellite') cells, in which the aged phenotype is a response to Wnt-signaling, can be activated to differentiate and regenerate muscle in aged animals as efficiently as young muscle stem cells — either by forced activation of Notch, or by factors in serum from young animals supplied by parabiosis [9,65–67]. Separately, attenuation of HSC aging could also be achieved by lifelong caloric restriction in BalbC inbred mice [68], or anti-oxidative therapy [69]. While these experiments involve different mechanisms, to date reported rational interventions that target intrinsic mechanisms of HSC aging are limited to the use of rapamycin and the Cdc42 activity inhibitor, CASIN [41••,70••]. While these are very encouraging results, additional research will be necessary to investigate to what extent such novel regeneration/rejuvenation pathways will attenuate aging-associated immunosenescense.

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