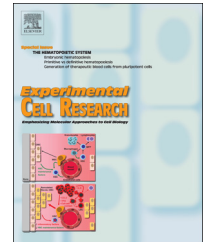


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Review Article

Regulation of hematopoietic stem cell aging by the small RhoGTPase Cdc42

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ABSTRACT / SUMMARY

Aging of stem cells might be the underlying cause of tissue aging in tissue that in the adult heavily rely on stem cell activity, like the blood forming system. Hematopoiesis, the generation of blood forming cells, is sustained by hematopoietic stem cells. In this review article, we introduce the canonical set of phenotypes associated with aged HSCs, focus on the novel aging-associated phenotype apolarity caused by elevated activity of the small RhoGTPase in aged HSCs, discuss the role of Cdc42 in hematopoiesis and describe that pharmacological inhibition of Cdc42 activity in aged HSCs results in functionally young and thus rejuvenated HSCs.

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Aging of stem cells limits the regenerative potential of tissues

The demographic development in most Western countries predicts that age-associated diseases and their prevention will become an important social, economic and medical topic. This trend in population ageing is unprecedented in human history, and the twenty-first century will witness even more rapid aging than did the century just past (<http://www.un.org/esa/population/publications/worldageing19502050>). Improved and in-depth knowledge of molecular and cellular mechanisms of aging is the basis for designing rational approaches and potential therapies to allow for healthy aging. Aging results in a gradual decline in the ability to maintain a cell or tissue function within an organism. 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.

Adult somatic stem cells were initially thought to be endowed with, per definition of the stem cell itself, unlimited self-renewal capacity, and thus exempt from aging. However, evidence accumulated over the past has found measurable and successive age-dependent decline in stem cell activity from adulthood to old age, including for example hematopoietic, intestinal and muscle stem cells. This age-associated decline in stem cell function leads to a decline in the regenerative capacity of the tissue, which might be in part contribute to mechanisms that limit lifespan. Skin, intestine and blood are composed of short-lived cells that require continuous replenishment by somatic stem cells to maintain tissue homeostasis. Current theory is therefore that especially aging of stem cells that form these tissues will greatly contribute to the decline in tissue function with aging, although such a view does not exclude that aging of stem cells in tissues with a low cellular turnover like the brain or even the heart might not at all contribute to reduced tissue homeostasis upon aging, for example via changes in secretory functions or niche-stem cells interactions. The underlying cellular and molecular mechanisms though of stem cell aging are still poorly identified, most likely due to the fact that stem cells are rare and thus require additional sophisticated experimental tools with respect to analyzing them via for example biochemical methods. Identifying mechanisms of stem cell aging and conditions under which aged stem cells become functionally similar to young stem cells might be important first steps towards devising treatments of aging-associated imbalance in tissue homeostasis and tissue regeneration with the ultimate goal of allowing for healthy aging.

Parameters of aged HSCs and aged hematopoiesis

HSCs from young and aged mice differ primarily in their function, which is also reflected in distinct molecular changes in phenotypically identified HSCs from young and aged mice and humans. Aged HSCs exhibit distinct whole genome expression signatures [1,2] and increased double-strand breaks as detected by increased levels of gammaH2AX staining, a surrogate marker for DNA double strand breaks, which though might be associated with replication stress upon aging [3–5]. HSC aging is driven by both intrinsic and extrinsic factors [1,6–10]. Due to the cell intrinsic component it is allowed to speak of young HSCs and aged HSCs

when speaking of HSCs from young and aged animals [11]. Aged HSCs show reduced self-renewal activity determined in serial transplant assays [12]. Aging has also a profound influence on the early differentiation patterns of HSCs. Many studies, including data from our laboratories, have demonstrated that aged HSCs are deficient in their ability to support erythropoiesis, and that aged HSCs do not efficiently generate T and B-lymphoid progeny while they are superior in supporting the myeloid cell lineage [13–15]. This difference in cell lineage self-renewal is emphasized by age-associated anemia and a decline in function of immune cells in aged individuals [1,16–22]. The lymphoid defect has been attributed to an impaired ability of aged HSCs to differentiate into the common-lymphoid progenitor cell, the progenitor cells that will give rise to both the T- and the B-cell lineage [1,23,24], while at the same time these CLPs from aged present with reduced proliferation potential [25]. Bone marrow of aged mice harbors thus far more myeloid restricted progenitor cells at the expense of lymphoid restricted progenitors compared to young mice [1]. This correlates with an overrepresentation of myeloid type cells and a diminution of either B-cells and/or T-cells in peripheral blood after transplantation of aged HSCs. In theory, this could result in a very effective innate immune response at the expense of a weak adaptive system in the aged. Unfortunately, as clinical wisdom [20] and initial experiments in animals [18,26,27] suggest, and similar to stem cells in aged C57BL/6 animals, the function of these myeloid type of progenitor cells is decreased, in lieu with the often weak innate response in aged individuals. What the underlying molecular mechanisms in this stem cell intrinsic shift in lymphoid/myeloid differentiation patterns are is an unsolved question, but it might be regulated by both stem cell intrinsic as well as extrinsic mechanisms. Recent evidence also suggests that the hematopoietic system might be maintained by a consortium of HSC subtypes (myeloid-biased HSCs and lymphoid-biased HSCs) that can be prospectively purified, and that aging changes the clonal composition of the HSC compartment because of a relative expansion of myeloid-biased HSCs (clonal diversity/expansion model) [28–31]. A commonly stated paradigm/assumption in the stem cell aging field is also that clonality, meaning the active number of clones contributing to hematopoiesis at a given time point, is reduced upon aging, although recent published data, tracking barcoded stem cells upon transplantation of aged HSCs, suggest the opposite, as in this work upon aging more clones with a smaller per clone contribution were identified [32,33]. Such findings will need to be reconfirmed, although in general they fit the model in which we have a clonal expansion of a cell lineage upon aging. Whether changes in clonality are linked to altered cell turnover or altered lineage determination has though, to the best of our knowledge, not been directly studied so far, but initial analyses suggest that both mechanisms might be at work [34]. Our own data showed 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 interaction with the microenvironment, while aged HSCs show enhanced mobilization from bone marrow to peripheral blood when induced by granulocyte-colony-stimulating factor [35–37]. Recently, changes in autophagy and metabolism have been suggested for aged HSCs, although additional data will be necessary to further validate these preliminary observations [38]. We have recently added a novel candidate mechanism of HSC aging to this list, the loss of cell polarity upon

aging [13]. Thus, a canonical set of features phenotypically separates young from aged HSCs. Aged HSCs therefore present with a compilation of phenotypes that change upon aging, and attenuation or rejuvenation efforts should aim at targeting most of these aging-associated changes of stem cell function.

The small RhoGTPase Cdc42 in hematopoiesis

Cdc42 belongs to the Rho GTPase family of the Ras superfamily, acting as a binary molecular switch that cycles between a GTP-bound active state and GDP-bound inactive state in response to a variety of extracellular stimuli [39]. A key function of Cdc42 is regulation of elements that structure cells like the actin cytoskeleton or part of the microtubule network, [40] which is believed to be a central mechanism for Cdc42-mediated cell polarization, adhesion and migration. Cdc42 is involved in induction of actin filopodia and microspikes – at least in part through the effectors PAK, WASP/N-WASP, IQGAP, and possibly the Par3/Par6 polarity protein complexes. Par3/Par6 complexes are for example known to regulate actin and tubulin cytoskeleton reorganization and cell polarity (referred to as planar cell polarity in some tissues) [41,42]. Another important function of Cdc42 is to regulate cell growth signaling. Cdc42 was found to crosstalk with the JAK-STAT pathway, and to be important for activation of Erk, JNK, p38 MAP kinases, and NF- κ B transcription [43–46]. Cdc42-regulated cell proliferation requires phosphorylation of the Cdc42 effector IQGAP1 with subsequent binding to Cdc42. The N-terminus of IQGAP1 interacts with mTOR. Thus, Cdc42 might couple cell growth and division via a Cdc42-IQGAP1-mTOR pathway [47]. Lagging behind the genetic studies in lower eukaryotes as well as cell lines and fibroblasts, genetic information of Cdc42 in mammalian physiology was limited until recently, in part due to an early lethality phenotype upon *cdc42* gene deletion in mouse embryos [48]. Because the conventional biochemical and cell biological approaches using dominant negative or constitutively active mutant over-expression impose a number of experimental limitations related to specificity, dosage and/or clonal variability, tissue-specific mouse gene targeting clearly represents an improved strategy in determining the functions of Cdc42 in vivo [49,50]. Recent studies employing the Cre/loxP mouse conditional knockout methodology have revealed a number of physiologically relevant, sometimes unexpected, functions of Cdc42, ranging from heart, pancreas, nervous system, blood, bone, eye, immune system, to the skin, in a tissue specific manner [50]. It is evident that Cdc42 has integral functions in many cell systems and achieves this by mediating signaling through multiple pathways. It is becoming clear that the function and signaling pathways regulated by Cdc42 are tissue and cell type-specific, and the general principles of Cdc42 function defined by in vitro methods or from one tissue cell type may or may not apply to another cell type in in vivo situations. This also means that observations for example from fibroblast (from which most of the information stems) might be informative for designing targeted experiments, but ultimately, in which pathway and function Cdc42 may operate in aged HSCs need to be stringently dissected in HSCs and cannot just be inferred from data obtained in other types of cells or systems [50]. The activity of Cdc42 is actually elevated upon aging in most of the tissues tested in the mouse [51], which initially implied a causative relationship within aging and Cdc42 activity.

Apolarity of HSCs—a novel aging-associated phenotype

Cell polarity is well characterized in epithelial cells (termed planar cell polarity, [52,53]) and neuronal stem cells, but was only recently described by us and others to also exist in HSCs [13,54,55]. Asymmetric distribution of cellular components upon division was found at single-cell resolution [56,57] and by a fluorescent Notch-activity indicator system [54,58,59]. Additional studies show that for example the loss of polarity in *Drosophila* germ-line stem cells correlates with reduced stemness function [60,61]. These data thus support a critical role of polarity in stem cell maintenance. Mechanisms of cell polarity establishment and maintenance are well characterized in epithelial cells and neuronal stem cells, but not in HSCs. Our data and other published reports imply both stem cell intrinsic and extrinsic influences on polarity determination in HSCs [13,59].

What might be the role of polarity in stem cells? Polarity can be associated with specialized and compartmentalized functions in HSCs, with migration or with division.

A defining feature of stem cells is their ability to maintain a balanced number of stem cells (self-renewal), while at the same time being able to generate specialized progeny (differentiation). Both processes depend on the ability of stem cells to undergo either symmetric or asymmetric divisions, which involve cell polarity [62–64]. One intensely debated paradigm holds that asymmetric distribution of cellular components at HSC division and, subsequently, in the daughter cells determines their fate. Such an asymmetric distribution of cellular components has been shown by single-cell immune-staining [56,57] and a fluorescent Notch-activity indicator system [58]. In stromal cells, cytokine distribution has been shown to correlate with cell fate determination [65]; and cytokines seem actually to be instructive in this process. Supporting a determining role for polarity in stem cell aging is a recent study showing that a loss of proper polarity in aged *Drosophila* germ-line stem cells correlates with their reduced function [60]. To date, the role of HSC polarity with respect to the mode of HSC division and cell fate potential of daughter cells has not been experimentally tested. Given the established role of Cdc42 in mediating morphologic polarity in many cell types and in regulating HSC differentiation, it has been somewhat logical to postulate that Cdc42 plays a role in coordinating polarity in HSCs.

A role for Cdc42 activity in hematopoietic stem cell aging and stem cell polarity

Genome-wide association studies of longevity in humans linked elevated expression of Cdc42 in hematopoietic cells to increased morbidity and aging [66]. We could show that animals with constitutively elevated Cdc42 activity in all tissues by genetic deletion of the negative regulator of Cdc42, Cdc42GAP, have a severe pre-mature aging syndrome in multiple tissues, including HSCs. Further, reduced adhesion to stroma and reduced stem cell polarity correlates with increased activity of Cdc42 in aged HSCs [35–37]. In aggregation, this work initially supported a novel concept that elevated Cdc42 activity and signaling play a role in HSC aging (Fig. 1).

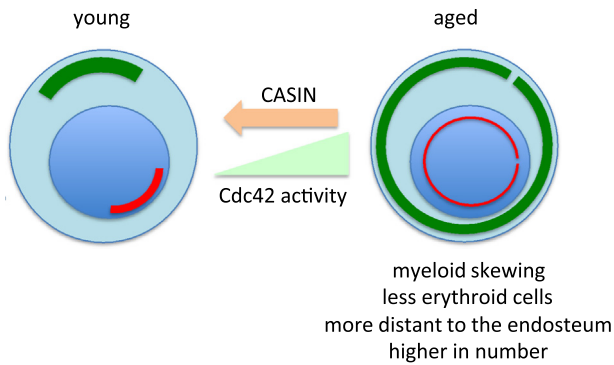


Fig. 1 – Cdc42 activity determines polarity and stem cell aging. Young stem cells are polar for cytoplasmic protein like tubulin (green) and nuclear protein like the acetylated form of histone 4 on lysine 16 (AcH4K16, red). Elevated Cdc42 activity found in aged HSCs turns these cells apolar for both tubulin and AcH4K16, which is tightly connected to aging-associated phenotypes of aged HSCs. Inhibition of Cdc42 activity to the level found in young mice via the specific inhibitor CASIN functionally rejuvenates aged HSCs and reverts apolarity in chronologically aged HSCs into polarity.

Rejuvenation of aged HSCs via inhibition of Cdc42 activity

The holy grail of aging research is the question of rejuvenation. Are molecular mechanisms of aging reversible? If elevated Cdc42 activity is causally linked to stem cell aging and apolarity, then reversion of the level to the level found in young HSCs might result in “younger” HSCs.

Aged HSCs exposed to a novel specific inhibitor of Cdc42 activity termed CASIN (Cdc42 activity-specific inhibitor (CASIN)) (5 μ M) in overnight *in vitro* cultures under low oxygen present with Cdc42 activity in aged HSCs to the level found in young HSCs (“normal level of activity”) and reverts chronologically aged, apolar HSCs into polar HSCs. Interestingly, the reversion of apolarity of aged HSCs upon CASIN treatment is stable even after CASIN is washed out, implying a non-reversible role of altered Cdc42 signaling in regulating HSC polarity in aged cells (like a memory effect). Such aged HSCs, in which Cdc42 activity level was, via pharmacological inhibition, reduced to the level found in young mice presented functionally and upon transplantation almost identically to young HSCs [13]. These data suggest that Cdc42 activity might represent a novel target to rejuvenate aged HSCs via altering stem cell polarity. Cdc42 activity regulated polarity is a valid surrogate marker for aged stem cells that can be pharmacologically targeted to rejuvenate aged HSCs.

Outlook

Pharmacological inhibition of Cdc42 activity is one promising possibility to alter aging associated phenotypes to rejuvenate HSCs, but other molecular and pharmacological approaches like inhibition of mTor signaling by rapamycin have been associated with stem cell rejuvenation [67]. Additional research will need to demonstrate whether such treatments can be translated to into healthy aging.

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