HHS Public Access

Author manuscript

Exp Cell Res. Author manuscript; available in PMC 2015 December 10.

Published in final edited form as:

Exp Cell Res. 2014 December 10; 329(2): 214–219. doi:10.1016/j.yexcr.2014.09.001.

Regulation of hematopoietic stem cell aging by the small RhoGTPase Cdc42

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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, disuccs 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.

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 ageing 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,

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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 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 suggest 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-κB

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 needs to be stringently dissected in HSCs and can not 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 plays a role in HSC aging.

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 μM) 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 a-polarity 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 HCS[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.

References

1. Rossi DJ, Bryder D, Zahn JM, Ahlenius H, Sonu R, Wagers AJ, Weissman IL. Cell intrinsic alterations underlie hematopoietic stem cell aging. Proc Natl Acad Sci U S A. 2005; 102:9194–9199. [PubMed: 15967997]
2. Chambers SM, Shaw CA, Gatza C, Fisk CJ, Donehower LA, Goodell MA. Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation. PLoS Biol. 2007; 5:e201. [PubMed: 17676974]
3. Rossi DJ, Bryder D, Seita J, Nussenzweig A, Hoeijmakers J, Weissman IL. Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. Nature. 2007; 447:725–729. [PubMed: 17554309]
4. Beerman I, Seita J, Inlay MA, Weissman IL, Rossi DJ. Quiescent hematopoietic stem cells accumulate DNA damage during aging that is repaired upon entry into cell cycle. Cell stem cell. 2014; 15:37–50. [PubMed: 24813857]
5. Flach J, Bakker ST, Mohrin M, Conroy PC, Pietras EM, Reynaud D, Alvarez S, Diolaiti ME, Ugarte F, Forsberg EC, Le Beau MM, Stohr BA, Mendez J, Morrison CG, Passegue E. Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells. Nature. 2014; 512:198–202. [PubMed: 25079315]
6. Geiger H, Van Zant G. The aging of lympho-hematopoietic stem cells. Nat Immunol. 2002; 3:329–333. [PubMed: 11919569]
7. Geiger H, Rennebeck G, Van Zant G. Regulation of hematopoietic stem cell aging in vivo by a distinct genetic element. Proc Natl Acad Sci U S A. 2005; 102:5102–5107. [PubMed: 15788535]
8. Ju Z, Jiang H, Jaworski M, Rathinam C, Gompf A, Klein C, Trumpp A, Rudolph KL. Telomere dysfunction induces environmental alterations limiting hematopoietic stem cell function and engraftment. Nat Med. 2007; 13:742–747. [PubMed: 17486088]
9. Rando TA. Stem cells, ageing and the quest for immortality. Nature. 2006; 441:1080–1086. [PubMed: 16810243]
10. Mayack SR, Shadrach JL, Kim FS, Wagers AJ. Systemic signals regulate ageing and rejuvenation of blood stem cell niches. Nature. 2010; 463:495–500. [PubMed: 20110993]
11. Geiger H, Rudolph KL. Aging in the lympho-hematopoietic stem cell compartment. Trends Immunol. 2009; 30:360–365. [PubMed: 19540806]
12. Janzen V, Forkert R, Fleming HE, Saito Y, Waring MT, Dombkowski DM, Cheng T, Depinho RA, Sharpless NE, Scadden DT. Stem-cell ageing modified by the cyclin-dependent kinase inhibitor p16(INK4a). Nature. 2006; 443:421–426. [PubMed: 16957735]
13. Florian MC, Dorr K, Niebel A, Daria D, Schrezenmeier H, Rojewski M, Filippi MD, Hasenberg A, Gunzer M, Scharffetter-Kochanek K, Zheng Y, Geiger H. Cdc42 activity regulates hematopoietic stem cell aging and rejuvenation. Cell stem cell. 2012; 10:520–530. [PubMed: 22560076]
14. Florian MC, Nattamai KJ, Dorr K, Marka G, Uberle B, Vas V, Eckl C, Andra I, Schiemann M, Oostendorp RA, Scharffetter-Kochanek K, Kestler HA, Zheng Y, Geiger H. A canonical to non-canonical Wnt signalling switch in haematopoietic stem-cell ageing. Nature. 2013; 503:392–396. [PubMed: 24141946]
15. Geiger H, de Haan G, Florian MC. The ageing haematopoietic stem cell compartment. Nat Rev Immunol. 2013; 13:376–389. [PubMed: 23584423]
16. Lipschitz DA. Age-related declines in hematopoietic reserve capacity. Semin Oncol. 1995; 22:3–5. [PubMed: 7863350]

17. Lipschitz DA, Udupa KB. Age and the hematopoietic system. J Am Geriatr Soc. 1986; 34:448–454. [PubMed: 3084611]
18. Butcher SK, Chahal H, Nayak L, Sinclair A, Henriquez NV, Sapey E, O'Mahony D, Lord JM. Senescence in innate immune responses: reduced neutrophil phagocytic capacity and CD16 expression in elderly humans. J Leukoc Biol. 2001; 70:881–886. [PubMed: 11739550]
19. Lord JM, Butcher S, Killampali V, Lascelles D, Salmon M. Neutrophil ageing and immunesenescence. Mech Ageing Dev. 2001; 122:1521–1535. [PubMed: 11511394]
20. Ginaldi L, De Martinis M, D'Ostilio A, Marini L, Loreto MF, Quaglino D. The immune system in the elderly: III. Innate immunity. Immunol Res. 1999; 20:117–126. [PubMed: 10580637]
21. Kim M, Moon HB, Spangrude GJ. Major age-related changes of mouse hematopoietic stem/progenitor cells. Ann N Y Acad Sci. 2003; 996:195–208. [PubMed: 12799297]
22. Sudo K, Ema H, Morita Y, Nakauchi H. Age-associated Characteristics of Murine Hematopoietic Stem Cells. J Exp Med. 2000; 192:1273–1280. Record as supplied by publisher. [PubMed: 11067876]
23. Allman D, Miller JP. The aging of early B-cell precursors. Immunol Rev. 2005; 205:18–29. [PubMed: 15882342]
24. Miller JP, Allman D. The decline in B lymphopoiesis in aged mice reflects loss of very early B-lineage precursors. J Immunol. 2003; 171:2326–2330. [PubMed: 12928378]
25. Min H, Montecino-Rodriguez E, Dorshkind K. Effects of aging on the common lymphoid progenitor to pro-B cell transition. J Immunol. 2006; 176:1007–1012. [PubMed: 16393987]
26. Plowden J, Renshaw-Hoelscher M, Engleman C, Katz J, Sambhara S. Innate immunity in aging: impact on macrophage function. Aging Cell. 2004; 3:161–167. [PubMed: 15268749]
27. Signer RA, Montecino-Rodriguez E, Witte ON, McLaughlin J, Dorshkind K. Age-related defects in B lymphopoiesis underlie the myeloid dominance of adult leukemia. Blood. 2007; 110:1831–1839. [PubMed: 17554060]
28. Challen GA, Boles NC, Chambers SM, Goodell MA. Distinct hematopoietic stem cell subtypes are differentially regulated by TGF-beta1. Cell stem cell. 2010; 6:265–278. [PubMed: 20207229]
29. Beerman I, Bhattacharya D, Zandi S, Sigvardsson M, Weissman IL, Bryder D, Rossi DJ. Functionally distinct hematopoietic stem cells modulate hematopoietic lineage potential during aging by a mechanism of clonal expansion. Proc Natl Acad Sci U S A. 107:5465–5470. [PubMed: 20304793]
30. Sieburg HB, Cho RH, Dykstra B, Uchida N, Eaves CJ, Muller-Sieburg CE. The hematopoietic stem compartment consists of a limited number of discrete stem cell subsets. Blood. 2005
31. Cho RH, Sieburg HB, Muller-Sieburg CE. A new mechanism for the aging of hematopoietic stem cells: aging changes the clonal composition of the stem cell compartment but not individual stem cells. Blood. 2008; 111:5553–5561. [PubMed: 18413859]
32. Verovskaya E, Broekhuis MJ, Zwart E, Weersing E, Ritsema M, Bosman LJ, van Poele T, de Haan G, Bystrykh LV. Asymmetry in skeletal distribution of mouse hematopoietic stem cell clones and their equilibration by mobilizing cytokines. J Exp Med. 2014; 211:487–497. [PubMed: 24567446]
33. Verovskaya E, Broekhuis MJ, Zwart E, Ritsema M, van Os R, de Haan G, Bystrykh LV. Heterogeneity of young and aged murine hematopoietic stem cells revealed by quantitative clonal analysis using cellular barcoding. Blood. 2013; 122:523–532. [PubMed: 23719303]
34. Dykstra B, Olthof S, Schreuder J, Ritsema M, de Haan G. Clonal analysis reveals multiple functional defects of aged murine hematopoietic stem cells. J Exp Med. 2011; 208:2691–2703. [PubMed: 22110168]
35. Xing Z, Ryan MA, Daria D, Nattamai KJ, Van Zant G, Wang L, Zheng Y, Geiger H. Increased hematopoietic stem cell mobilization in aged mice. Blood. 2006; 108:2190–2197. [PubMed: 16741255]
36. Geiger H, Koehler A, Gunzer M. Stem Cells, Aging, Niche, Adhesion and Cdc42: A Model for Changes in Cell-Cell Interactions and Hematopoietic Stem Cell Aging. Cell Cycle. 2007; 6
37. Kohler A, Schmithorst V, Filippi MD, Ryan MA, Daria D, Gunzer M, Geiger H. Altered cellular dynamics and endosteal location of aged early hematopoietic progenitor cells revealed by time-lapse intravital imaging in long bones. Blood. 2009; 114:290–298. [PubMed: 19357397]

38. Kohli L, Passegue E. Surviving change: the metabolic journey of hematopoietic stem cells. Trends Cell Biol. 2014; 24:479–487. [PubMed: 24768033]
39. Etienne-Manneville S, Hall A. Rho GTPases in cell biology. Nature. 2002; 420:629–635. [PubMed: 12478284]
40. Nobes CD, Hall A. Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. Cell. 1995; 81:53–62. [PubMed: 7536630]
41. Bishop AL, Hall A. Rho GTPases and their effector proteins. Biochem J. 2000; 348(Pt 2):241–255. [PubMed: 10816416]
42. Kesavan G, Sand FW, Greiner TU, Johansson JK, Kobberup S, Wu X, Brakebusch C, Semb H. Cdc42-mediated tubulogenesis controls cell specification. Cell. 2009; 139:791–801. [PubMed: 19914171]
43. Lamarche N, Tapon N, Stowers L, Burbelo PD, Aspenstrom P, Bridges T, Chant J, Hall A. Rac and Cdc42 induce actin polymerization and G1 cell cycle progression independently of p65PAK and the JNK/SAPK MAP kinase cascade. Cell. 1996; 87:519–529. [PubMed: 8898204]
44. Coso OA, Chiariello M, Yu JC, Teramoto H, Crespo P, Xu N, Miki T, Gutkind JS. The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. Cell. 1995; 81:1137–1146. [PubMed: 7600581]
45. Minden A, Lin A, Claret FX, Abo A, Karin M. Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. Cell. 1995; 81:1147–1157. [PubMed: 7600582]
46. Perona R, Montaner S, Saniger L, Sanchez-Perez I, Bravo R, Lacal JC. Activation of the nuclear factor-kappaB by Rho, CDC42, and Rac-1 proteins. Genes Dev. 1997; 11:463–475. [PubMed: 9042860]
47. Wang JB, Sonn R, Tekletsadik YK, Samorodnitsky D, Osman MA. IQGAP1 regulates cell proliferation through a novel CDC42-mTOR pathway. J Cell Sci. 2009; 122:2024–2033. [PubMed: 19454477]
48. Mulloy JC, Cancelas JA, Filippi MD, Kalfa TA, Guo F, Zheng Y. Rho GTPases in hematopoiesis and hemopathies. Blood. 2010; 115:936–947. [PubMed: 19965643]
49. Melendez J, Grogg M, Zheng Y. Signaling role of Cdc42 in regulating mammalian physiology. J Biol Chem. 2011; 286:2375–2381. [PubMed: 21115489]
50. Wang L, Zheng Y. Cell type-specific functions of Rho GTPases revealed by gene targeting in mice. Trends Cell Biol. 2007; 17:58–64. [PubMed: 17161947]
51. Wang L, Yang L, Debidda M, Witte D, Zheng Y. Cdc42 GTPase-activating protein deficiency promotes genomic instability and premature aging-like phenotypes. Proc Natl Acad Sci U S A. 2007; 104:1248–1253. [PubMed: 17227869]
52. Humbert PO, Dow LE, Russell SM. The Scribble and Par complexes in polarity and migration: friends or foes? Trends Cell Biol. 2006; 16:622–630. [PubMed: 17067797]
53. Martin-Belmonte F, Perez-Moreno M. Epithelial cell polarity, stem cells and cancer. Nat Rev Cancer. 2012; 12:23–38. [PubMed: 22169974]
54. Ting SB, Deneault E, Hope K, Cellot S, Chagraoui J, Mayotte N, Dorn JF, Laverdure JP, Harvey M, Hawkins ED, Russell SM, Maddox PS, Iscove NN, Sauvageau G. Asymmetric segregation and self-renewal of hematopoietic stem and progenitor cells with endocytic Ap2a2. Blood. 2012; 119:2510–2522. [PubMed: 22174158]
55. Giebel B. Cell polarity and asymmetric cell division within human hematopoietic stem and progenitor cells. Cells Tissues Organs. 2008; 188:116–126. [PubMed: 18160821]
56. Rajendran L, Beckmann J, Magenau A, Boneberg EM, Gaus K, Viola A, Giebel B, Illges H. Flotillins are involved in the polarization of primitive and mature hematopoietic cells. PLoS ONE. 2009; 4:e8290. [PubMed: 20027317]
57. Beckmann J, Scheitza S, Wernet P, Fischer JC, Giebel B. Asymmetric cell division within the human hematopoietic stem and progenitor cell compartment: identification of asymmetrically segregating proteins. Blood. 2007; 109:5494–5501. [PubMed: 17332245]

58. Wu M, Kwon HY, Rattis F, Blum J, Zhao C, Ashkenazi R, Jackson TL, Gaiano N, Oliver T, Reya T. Imaging hematopoietic precursor division in real time. Cell stem cell. 2007; 1:541–554. [PubMed: 18345353]

59. Ito K, Carracedo A, Weiss D, Arai F, Ala U, Avigan DE, Schafer ZT, Evans RM, Suda T, Lee CH, Pandolfi PP. A PML-PPAR-delta pathway for fatty acid oxidation regulates hematopoietic stem cell maintenance. Nat Med. 2012; 18:1350–1358. [PubMed: 22902876]

60. Cheng J, Turkel N, Hemati N, Fuller MT, Hunt AJ, Yamashita YM. Centrosome misorientation reduces stem cell division during ageing. Nature. 2008; 456:599–604. [PubMed: 18923395]

61. Peng Y, Axelrod JD. Asymmetric protein localization in planar cell polarity: mechanisms, puzzles, and challenges. Curr Top Dev Biol. 2012; 101:33–53. [PubMed: 23140624]

62. Morrison SJ, Kimble J. Asymmetric and symmetric stem-cell divisions in development and cancer. Nature. 2006; 441:1068–1074. [PubMed: 16810241]

63. Schroeder T. Asymmetric cell division in normal and malignant hematopoietic precursor cells. Cell stem cell. 2007; 1:479–481. [PubMed: 18938740]

64. Takano H, Ema H, Sudo K, Nakauchi H. Asymmetric division and lineage commitment at the level of hematopoietic stem cells: inference from differentiation in daughter cell and granddaughter cell pairs. J Exp Med. 2004; 199:295–302. [PubMed: 14744992]

65. Rieger MA, Schroeder T. Instruction of lineage choice by hematopoietic cytokines. Cell Cycle. 2009; 8:4019–4020. [PubMed: 19949300]

66. Kerber RA, O'Brien E, Cawthon RM. Gene expression profiles associated with aging and mortality in humans. Aging Cell. 2009; 8:239–250. [PubMed: 19245677]

67. Chen C, Liu Y, Zheng P. mTOR regulation and therapeutic rejuvenation of aging hematopoietic stem cells. Sci Signal. 2009; 2:ra75. [PubMed: 19934433]

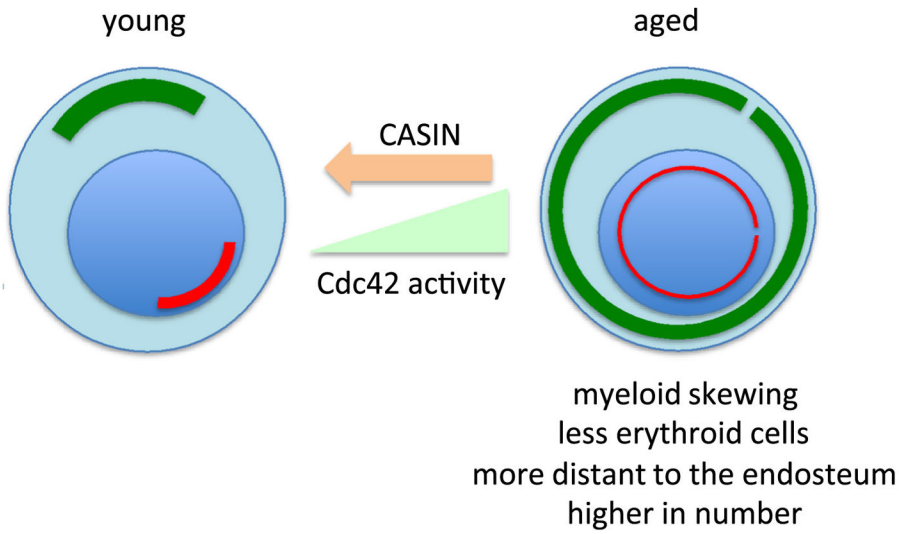

Figure 1. Cdc42 activity determines polarity and stem cell aging

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.