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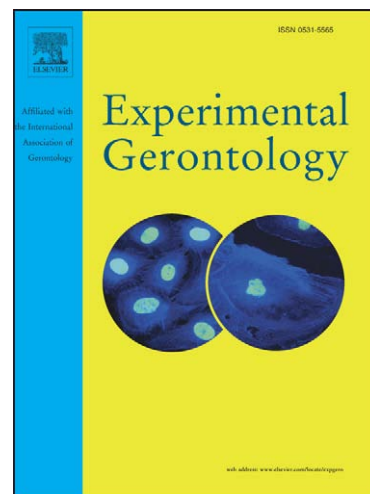
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AGING OF HEMATOPOIETIC STEM CELLS IS REGULATED BY THE STEM CELL NICHE

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Abstract

Adult stem cells provide the basis for regeneration of aging tissue. Their dual ability for self-renewal and multilineage differentiation is controlled by direct interaction with a specific microenvironment – the so called “stem cell niche”. Hematopoietic stem cells (HSC) reside in the bone marrow. It is still under debate if HSC can rejuvenate infinitely or if they do not possess “true” self-renewal and undergo replicative senescence such as any other somatic cell. Furthermore, the question arises to what extent age-related changes in HSC are due to intrinsic factors or regulated by external stimuli. There is growing evidence, that the stem cell niche is most important for the regulation of cellular aging in adult stem cells. It is the stem cell niche that (i) maintains HSC in a quiescent state that reduces DNA damage as well as replicative senescence, (ii) protects from radicals and toxic compounds, (iii) regulates cell intrinsic signal cascades and (iv) modulates gene expression and epigenetic modifications in HSC. Thus, the interplay with the stem cell niche controls HSC function including the aging process of the hematopoiesis.

Stem cells and aging

Aging is a complex process that involves every cell and organ in the body and that leads to the deterioration of many body functions over the lifespan of an individual. The reduced capacity to regenerate injured tissues or organs and an increased propensity to infections and cancers are probably the most prominent features of aging. Adult stem cells such as hematopoietic stem cells (HSC) or mesenchymal stem cells (MSC) assure lifelong regeneration of adult tissues (e.g. all different types of blood cells or bone, fat and cartilage respectively). If the rejuvenating effect of stem cells was perfect, senescent cells could be replaced indefinitely. In the context of time and aging, stem-cell research is important for two reasons. On the one hand, stem cells are a good model to study aging as they provide the basis for regeneration of aging tissue. On the other hand, finding ways to reactivate stem cells and control their target destination might create unforeseen opportunities for treatment of degenerative diseases. Hence, all aging phenomena can be interpreted as signs of aging at the level of somatic stem cells - a human body is as old as its stem cells (Ho et al., 2005).

All normal somatic cells in mammals stop proliferation after a certain number of cell divisions *in vitro* before they enter a state called senescence. Senescent cells are viable and metabolically active but no longer have the ability to proliferate and this is in contrast to reversible forms of cell cycle arrest such as quiescence. Since the first description of replicative senescence, it has been speculated that this process might also govern aging of the whole organism. Two fundamental ways have been hypothesized how this process may be governed: Aging might either be the result of a purposeful program driven by genes or rather be evoked by stochastic or random, accidental events (Hayflick, 2007). Progressive shortening of the telomeres or modified telomeric structures is thought to be a main trigger for replicative senescence. Telomerase activity in normal human cells is generally undetectable leading to successive erosion of telomeres with each cell division. In contrast germline cells, embryonic stem cells (ESC) and adult stem cells maintain their telomeres by activated telomerase and this counteracts replicative senescence. However, this mechanism

is unlikely to be the only cause of this phenomenon (O'Hare et al., 2001; Di Donna et al., 2003). There is also evidence that senescence involves DNA damage and oxidative stress (Kiyono et al., 1998; Ho et al., 2005). Clearly, cellular senescence is a complex process and the sequence of its molecular events is thus far unknown.

In general, adult stem cells have a slow turnover and reside in specialized niches, protected from the environment and only a few are activated at a time (Wilson and Trumpp, 2006). Most of the long-term self-renewing HSC remain in G0 phase in contrast to transient amplifying progenitors cells (Cheshier et al., 1999). The stem cell niche governs asymmetric cell division of two daughter cells that may either self-renew to replenish the stem cell pool or to differentiate to more committed cells (Ho and Wagner, 2007). In addition the stem cell niche might also play a crucial role in regulation of aging. In this review we will discuss how aging of HSC might be influenced and regulated by the microenvironment.

Self-renewal and differentiation – the hallmark of stem cells

Modern stem cell research began in 1963 when Siminovitch et al. proved the existence of hematopoietic stem cells (HSC) in the bone marrow (BM) of mice (Siminovitch et al., 1963). In series of experiments they showed that cells from the bone marrow could reconstitute hematopoiesis and hence rescue lethally irradiated recipient animals. Furthermore, by serial transplantations, they demonstrated self-renewal ability of the original bone marrow cells. Based on these experiments HSC were defined by their capability for self-renewal to maintain the stem cell pool as well as multilineage differentiation. This dual function is still considered as the hallmark for adult stem cells. The question remains however, if self-renewal of stem cell function does also imply rejuvenation or if stem cells undergo the process of aging such as any other somatic cell.

Hematopoietic stem cells – loss or accumulation with aging?

Analysis of aging in HSC is limited by two hurdles: the available methods for their isolation as well as by the problem to precisely define their functional characteristics. The cell surface glycoprotein CD34 is used as a surrogate marker for human HSC. The majority of CD34⁺ cells however comprise more committed progenitor cells and HSC can be further enriched in the CD34⁺/CD38⁻ cell fraction. In the murine system a variety of further markers have been described such as c-Kit⁺/Thy1.1^{lo}/Sca-1⁺/Lin⁻ (KTSL) or a combination of signaling lymphocyte activation molecules (SLAM) receptors (CD150⁺/CD244⁻/CD48⁻ or CD150⁺/CD48⁻/CD41⁻) (Kiel et al., 2005). Despite these efforts it is so far not possible to precisely define a pure population of primitive HSC with repopulation ability. Demonstration of intrinsic changes, cellular-senescence or numeric changes in HSC during the process of aging is therefore limited by cell fractions enriched in HSC and this might be a reason for contradictory observations between different studies (Dykstra and de Haan, 2008).

Furthermore, analysis of the aging process in stem cells is limited by the available methods for functional characterization. Various *in vitro* assays provide an estimate for the number and maintenance of primitive hematopoietic progenitor cells (HPC) such as colony-forming unit (CFU) assay, cobblestone area forming cell (CAFC) assay, long-term culture-initiating cell (LTC-IC) assay or multilineage initiating cell (ML-IC) assay (de Wynter and Ploemacher, 2001). Murine *in vivo* transplantation models such as competitive repopulation unit (CRU) assay or SCID-repopulating cell (SRC) assay represent a more authentic test system for engraftment and differentiation potential. On the other hand, transplantation assays are very complex, the immune system of these mice is altered and there are significant differences between different mouse strains. Finally, mice may not be an adequate model organism for the human stem cell niche. Each of these assays has its advantages and disadvantages and the results of two different assays do not necessarily correlate with each other.

Despite these limitations recent data indicated that the numbers of HSC increase with age. This was unexpected, as it has been anticipated that aging may be caused by the depletion of stem and progenitor cells and therefore reduced regeneration potential. Competitive repopulation assays in murine transplantation models demonstrated that older bone marrow contains a higher number of repopulating HSC (2-3 fold higher) (Morrison et al., 1996). This is in line with similar *in vitro* experiments using the cobblestone area forming cell (CAFC) assay as a surrogate assay for HSC that demonstrated that the number of HSC increases about four-fold with age (de Haan and Van Zant, 1999; Dykstra and de Haan, 2008). On the other hand, there is evidence that the increase in HSC number comprises mainly progenitor cells whereas a loss of more primitive HSC occurs (Dykstra and de Haan, 2008). Various studies have indicated that even though similar HSC concentrations could be found in young and old bone marrow, it is the functional ability per cell in the repopulation model that shows a significant reduction with increasing donor age (Chen, 2004). It has been described that HSC from older mice have a significantly lower cycling activity than those isolated from young mice (de Haan et al., 1997). Furthermore, age-related changes in HSC number in murine models are highly strain-dependent (Dykstra and de Haan, 2008). Whereas the impact of aging on the quantity and quality of murine HSC has been recently studied, information on age-related changes in human HSC is scarce. Studying this is also a challenge because these cells can maintain their function much longer than the average human lifespan. It needs to be demonstrated if the knowledge obtained from murine transplantation models can be extrapolated to the human system.

Aging of the stem cell niche

Various intrinsic programs and pathways facilitate the unique characteristics of stem cells. These programs regulate maintenance, proliferation and differentiation of HSC but they have to be tightly controlled by the microenvironment according to the physiological needs of the organism. 1978 Schofield proposed a hypothesis in which stem cells are seen in association with other cells which determine their behavior – the so called “stem cell niche” (Schofield,

1978). For hematopoietic stem cells two types of niches have been identified in the bone marrow: the endosteal niche and the vascular niche. There is evidence that the most primitive HSC are located close to the endosteum of the bone in proximity to stromal cells and osteoblasts. Presumably the reciprocal communication and adhesion between these cell types regulate stem cell function (Zhang et al., 2003; Jones and Wagers, 2008). HSC are also found in the vicinity of sinusoidal endothelial cells, which are referred to as the vascular niche. It has been suggested that the vascular niche forms a milieu that supports proliferation, differentiation and trans-endothelial migration of HSC, whereas the endosteal niche supports quiescence and self-renewal (Sugiyama et al., 2006; Geiger et al., 2007).

The precise mechanism of this interaction of HSC with their local environment is largely unknown. However, it is certain, that the composition of cell types and extracellular matrix components in the bone marrow varies extensively during lifetime. These changes are reflected by decreased bone formation and loss of bone mass and they are more pronounced in patients with osteoporosis. This is in conjunction of enhanced adipogenesis and down-regulation of osteoblastogenesis (Justesen et al., 2001). Furthermore, the composition of the mature hematopoietic cells changes during lifetime. Especially the immune system is affected by aging: the lymphoid differentiation potential in bone marrow of elderly donors is significantly reduced (Linton and Dorshkind, 2004). Transplantation experiments indicated that this myeloid skewing of the differentiation potential with age is probably due to intrinsic changes in older HSC (Kim et al., 2003; Dykstra and de Haan, 2008). Furthermore recent studies indicate that aging is not only associated with functional alterations of HSC but also with an altered microenvironment that is required for hematopoietic differentiation. HSC from young mice had a reduced differentiation potential towards lymphocyte phenotype in aged than in young recipients. On the other hand, HSC from aged mice also had a reduced lymphoid differentiation potential in young mice (Li et al., 2001; Donnini et al., 2007; Zhu et al., 2007). These studies indicate that aging is associated with HSC intrinsic changes as well as with changes in the microenvironment. Taken together,

the stem cell niche undergoes extensive changes during the lifetime of the organism and these might play an important role for external control of stem cell aging.

Potential mechanisms involved in aging of hematopoietic stem cells

Central questions in adult stem cells and their function in the context of aging are yet unravelled: In theory, HSC might either have the unlimited potential for self-renewal including rejuvenation or they might undergo cellular aging such as other somatic cells. Furthermore, the question arises to what extent age-related changes in these cells are due to intrinsic factors or regulated by stimuli from the microenvironment – the stem cell niche. Four possible scenarios will be described in the following paragraph and the truth lies most likely somewhere between these (figure 1).

1) HSC can rejuvenate infinitely

“True” self-renewal of stem cells indicates that the daughter cell possesses the same molecular features and functional abilities as the mother cell and this should also include their rejuvenation. This would enable infinite maintenance of the stem cell pool. If this was the case, adult stem cells could also provide an infinite basis for tissue regeneration. In this context, the continuous loss or regenerative potential of aging tissue might be attributed to a reduction in the number of primitive stem cells. Proof of this thesis is hampered by the crude functional measurement of HSC function that would need to discriminate between true stem cells and progenitor cells. Furthermore, demonstration that the daughter cells share precisely the same molecular features as their parent cells is not possible due to the fact that the parent cell is no more existent after cell division (Dykstra and de Haan, 2008). Thus, the theory of infinite rejuvenation of HSC as it is observed in germline stem cells or ESC can hardly be verified.

2) HSC undergo cellular aging

In contrast to the above mentioned thesis HSC could undergo replicative senescence such as all mammalian cells. In this case there is no “true” self-renewal as the daughter cells may have the same multilineage differentiation potential but after a limited number of cell cycles the daughter cells enter a senescent state and stop proliferation. In addition to the above mentioned limitations in HSC research analysis of replicative senescence is further hampered by the absence of reliable *in vitro* expansion protocols for primitive HSC. For mesenchymal stem cells (MSC) culture expansion protocols are established and our group has recently demonstrated that replicative senescence in these cells is a continuous and organized process (manuscript in revision). However, MSC are only poorly defined on a molecular level and verification of their stem cell function is not unequivocally demonstrated (Wagner and Ho, 2007). Exhaustion of the HSC pool does not normally occur during lifetime. Serial transplantation experiments in the murine model demonstrated that the declining repopulation ability and exhaustion of HSC can be protracted for more than five rounds of transplantation (Harrison and Astle, 1982). Despite a remarkable proliferative capacity these results indicate cellular aging of HSC. Mathematical models for HSC differentiation demonstrate that the Hayflick hypothesis is compatible with lifelong hematopoiesis (Dingli and Pacheco, 2006).

Furthermore, replicative senescence in HSC is supported indirectly by the fact that most of them remain in a quiescent state in the bone marrow. Primitive HSC can even be enriched in the slow dividing fraction (SDF) of CD34⁺/CD38⁻ cells. In our previous work we have demonstrated that asymmetric cell division of primitive HSC correlates with asymmetric cell division kinetics (Wagner et al., 2004; Giebel et al., 2006). One daughter cell remains quiescent or divides very slowly while the other multiplies exponentially to yield committed progenitors and lineage specific colonies. If the two daughter cells of slow dividing cells were separated by micromanipulation and analyzed individually, it has been demonstrated that one daughter cell inherited the developmental capacity of the mother cell, whereas the other

one became more specified (Giebel et al., 2006). We have exploited differences in division kinetics to separate the CD34⁺/CD38⁻ cells into a SDF and a committed, fast-dividing fraction (FDF). Several molecular markers associated with primitive stem cell function are higher expressed in the SDF compared to the FDF (Wagner et al., 2004). The underlying reason for quiescence or slow cell division might either be protection from mutations during DNA replication or prevention of replicative exhaustion of stem cells.

3) Cellular aging is regulated by the microenvironment

A number of studies have demonstrated that hematopoiesis is affected by the aging of the hematopoietic microenvironment. HSC have a reduced ability to home to their niches upon transplantation into old *versus* young recipients (Rossi et al., 2005; Liang et al., 2005). Furthermore, adhesive interaction of HSC with their niche is modulated by age-related alterations as aged mice show an increased mobilization of HSC in response to granulocyte-colony-stimulating factor (G-CSF) (Xing et al., 2006). Recently Ju et al. have demonstrated that telomere dysfunction exerts especially effects on the hematopoietic microenvironment. In telomerase knockout mice B-lymphopoiesis is impaired whereas myelopoiesis is increased. Furthermore telomere dysfunction and aging limited the engraftment of transplanted wild-type HSC (Ju et al., 2007). These results provide evidence, that the supportive function or the size of the stem cell niche is modified during the progress of aging.

4) The microenvironment keeps HSC in a quiescent state to prevent replicative senescence

Whereas HSC are kept in a quiescent state under normal conditions they have to be activated to enhance hematopoiesis according to the physiological needs, especially after chemotherapy. Cell division, self-renewal and differentiation are tightly controlled by the stem cell niche (Ho and Wagner, 2007). Keeping HSC in a quiescent state might reduce the number of mutations that could ultimately lead to the development of leukemia. On the other hand this might delay exhaustion of primitive HSC by replicative senescence. If HSC undergo replicative senescence, then aging is modulated indirectly by external stimuli of the stem cell niche that regulate their proliferation.

Stromal cells as a model system for the stem cell niche

In 1973 Dexter and coworkers described that by co-cultivation of hematopoietic bone marrow cells with thymus cells it was possible to maintain different stages of granulocytic cells for at least ten weeks (Dexter et al., 1973). These early experiments demonstrated that feeder layer cells may provide a supportive cellular environment to maintain primitive function of hematopoietic cells. Although numerous studies have demonstrated the vital role of stromal feeder layers for maintenance of multi-potency of HPC *in vitro*, little is known about the precise cellular and molecular mechanisms of this interaction. HPC are characterized by rapid migratory activity and constantly changing morphology. They actively migrate towards stromal cells and extend magnupodia in the direction of the latter (Wagner et al., 2005). The essential role of direct cell-cell contact for the regulation of self-renewal and differentiation of adult stem cells has been shown in various organisms and cell systems. Specific junctional complexes may play a similar role in the hematopoietic system. Various adhesion proteins have been implicated in this interaction such as N-cadherin, integrin beta-1 (CD29), VCAM1, NCAM1, notch and CD44 (Wagner et al., 2007a; Wagner et al., 2007b) and further studies will be necessary to determine the relative significance of these cell adhesion proteins. This represents an artificial model system that does not take into account the complex composition of cellular and extracellular components of the stem cell niche. However, it may help to further elucidate the molecular mechanisms involved in the regulation of stem cell function including its influence on cellular aging. Supporting this concept, it has been demonstrated that stromal cells from younger bone marrow have a higher hematopoiesis supportive activity compared to those from old bone marrow samples (Mauch et al., 1982). Furthermore, we observed a lower proliferation rate on hematopoiesis supportive feeder layer cells in comparison to non-supportive MSC from adipose tissue and these results indicate, that supportive stromal cells keep HPC in a quiescent state (Wagner et al., 2007a). Finding a method for culture expansion of primitive HSC would be of high interest for stem cell biology and for clinical application. So far *ex vivo* expansion of HSC resulted prevailing in

expansion of mature progenitors rather than immature HSC and this might partly be due to senescence of HSC under *in vitro* conditions. A better understanding of the interaction between HSC and the stem cell niche and of the mechanisms involved in regulation of stem cell aging might pave the way for reliable expansion of HSC.

Stem cell aging is regulated by the microenvironment

As mentioned above, there is evidence that the microenvironment influences aging of HSC. The precise molecular mechanisms are still unknown. Various effectors for aging have recently been described and some of these might also be subjected to external control by the stem cell niche (figure 2). The following examples show that age related changes of hematopoiesis are a result of intrinsic alterations in the HSC themselves as well as of the hematopoietic microenvironment that regulates and maintains stem cell function.

Progressive loss of telomeres

The successive shortening of telomere length with every cell division is one of the best described mechanisms involved in cellular aging. Telomeres are elongated by the enzyme telomerase. Absence of telomerase results in persistent telomere shortening and finally loss of telomere function, which is associated with replicative senescence or apoptosis. Stem cells normally express telomerase in contrast to most somatic cells. However, in HSC telomerase activity is low and thus telomere shortening still occurs during aging and limits their proliferative lifespan (Allsopp et al., 2003a). On the other hand, in a serial mouse transplantation model with telomerase over-expression HSC of both transgenic and non-transgenic animals could be serially transplanted no more than four times (Allsopp et al., 2003b). These results indicate that other mechanisms can limit stem cell function as well. Furthermore, telomere dysfunction impairs also the stem cell niche. Using telomerase knockout mice (*Terc*^{-/-}) Ju and coworkers demonstrated that telomerase dysfunction induces alterations in the bone marrow microenvironment by diminishing the stromal cell compartment and reducing the capacity of bone marrow stromal cells in their hematopoiesis

supportive activity (Ju *et al.*, 2007). The *Terc*^{-/-} dysfunctional environment limited the engraftment of transplanted wild-type HSC. The reduced hematopoiesis supportive activity was age dependent and correlated with progressive telomere shortening in bone marrow stromal cells. Furthermore, telomere dysfunction altered the expression of various cytokines in the plasma of aging *Terc*^{-/-} mice (Ju *et al.*, 2007). These results provide clear evidence, that telomere shortening is neither the unique nor an exclusively intrinsic mechanism for cellular aging in HSC. In contrast the loss of telomeres induces age dependent alterations in the stem cell environment that can impair the function and engraftment of HSC.

Reactive oxygen species and other toxic compounds

Aging of HSC might also be attributed to a continuous accumulation of cellular defects for example by damaging compounds such as reactive oxygen species (ROS). ROS arise as by-products of numerous metabolic processes and it causes direct damage to proteins, membranes and DNA. The oxidative stress mediates activation of several stress induced proteins as ERK, p38 MAPK, or p53. Glycation of proteins provides another harmful mechanism that leads to cross-linked proteins (Finkel and Holbrook, 2000). Over time, these proteins as well as oxidant-damaged DNA accumulate and eventually disrupt cellular function. In this context it is intriguing to note, that HSC in their stem cell niche are predominantly located at the lowest end of an oxygen gradient in the bone marrow. There is experimental evidence that that regionally defined hypoxia plays a fundamental role in regulating stem cell function (Fehrer *et al.*, 2007; Parmar *et al.*, 2007). The protection of the stem cell niche from harmful compounds might play a central role in prevention of HSC aging.

Regulation of gene expression

Recent studies comparing gene expression profile of purified HSC from young and old mice showed up-regulation of genes involved in inflammatory and stress within age (Chambers *et al.*, 2007). Whereas the functional role of these genes has been demonstrated it is yet unknown how they are regulated in the process of aging and to which degree the

microenvironment is involved in this mechanism. Co-cultivation of HSC with stromal cells provides a good model to get a first idea about the influence of the stem cell niche on the gene expression profile of HSC. We have previously described that a multitude of genes was differentially expressed in CD34⁺/CD38⁻ HPC upon interaction with stromal cells. Among the genes up-regulated were many involved in regulation of cell division, reorganization of the cytoskeleton and maintenance of genetic stability (Wagner et al., 2005). Therefore, gene expression of HSC is not a purely intrinsic process but it is regulated by the interplay of different cell types of the stem cell niche.

Regarding the gene expression involved in aging of stem cells we have recently observed continuous changes in the expression of several miRNAs in the process of replicative senescence in MSC (manuscript in revision). Mature miRNAs constitute a group of endogenous small, non coding RNAs of approximately 22 nucleotides in length that are expressed in a tissue- and cell-type specific manner and play essential roles in development. These single miRNA molecules, cleaved from 70-100 nucleotide hairpin pre-miRNA precursors exert a post-transcriptional effect on gene expression (Bartel, 2004) and some of these might be involved in the process of aging. For the nematode worm *Caenorhabditis elegans* it was demonstrated that over-expression of lin-4 led to extended life span, and an overall age-related decline in miRNA expression was described (Boehm and Slack, 2005; Ibanez-Ventoso et al., 2006). For the MSCs we assume that the up-regulation of specific miRNAs has functional implications for cellular aging and this will be addressed in the future.

Regulation of epigenetic modifications

Epigenetic mechanisms such as DNA methylation or histone acetylation regulate gene transcription at the chromatin level. As these modifications can be inherited by the daughter cells they have been demonstrated to play key roles in altering the long-term fate of HSC and hence, they might also be involved in the regulation or stem cell aging (Ruau et al., 2008).

Gene expression analysis of HSC from old mice compared with those from young mice revealed that genes involved in chromatin remodelling and transcriptional silencing were found to be down-regulated. Furthermore, inapposite expression of genes normally regulated by epigenetic mechanisms was observed (Chambers et al., 2007; Rossi et al., 2007a). Culture-induced decline of human HSC can be diminished by treatment with DNA methylation or histone acetylation interfering drugs whereas self-renewal is increased (Young et al., 2004; Bug et al., 2005). Epigenetic modifications in the process of aging might either be due to a loss of epigenetic regulation that may drive functional attenuation of HSC or to a purposeful mechanism where changes can be transmitted to progeny. We have previously demonstrated, that various genes including DNA (cytosine-5-)-methyltransferase 1 (dnmt1) were up-regulated upon contact between CD34⁺/CD38⁻ HPC with stromal cells and this indicates that intrinsic epigenetic modifications can be modulated by cell-cell interaction with the microenvironment (Wagner et al., 2005).

DNA damage accumulation during aging

Accumulation of endogenous DNA mutations is another key mechanisms that contributes to aging of HSC (Kenyon and Gerson, 2007). Mouse experiments have revealed that aged HSC show a higher extend of DNA damage (Rossi et al., 2007a). Additionally genes involved in DNA repair and maintenance were found to be down-regulated in HSC from old mice compared with those from young mice (Chambers et al., 2007; Rossi et al., 2007a). Furthermore, mice that were deficient for genes involved in DNA repair or maintenance show premature hematopoietic exhaustion (Rossi et al., 2007a). These data indicate that DNA repair and genomic maintenance play a key role in stem cell aging. This effect might be attenuated in HSC as a result of their quiescent (G0) cell cycle status, which results in an attenuation of checkpoint control and DNA damage in the context of DNA replication (Rossi et al., 2007b). As mentioned above, maintenance in a quiescent state and proliferation are governed by the stem cell niche and this seems to be another important mechanism for external modulation of cellular aging.

Conclusion

Cellular aging and senescence does not necessarily represent an inevitable fate for all cells, because cellular senescence is not observed in primitive organisms such as sponges or corals as well as in our germline cells. Aging in different species correlates with the generation time and it seems to provide an evolutionary advantage for the whole species. It is intriguing to note, that this process is restricted to higher organisms with a mesoderm and highly specialized stem cell niches. External control by the microenvironment might be more robust with respect to mutations – hereby it is not a unique stem cell that might gather a growth advantage and lead to tumor formation. Recent data suggest that we age, in part, because our self-renewing stem cells grow old as a result of heritable intrinsic events, such as DNA damage or replicative senescence. On the other hand there is emerging evidence, that extrinsic control by the stem cell niche plays a central role in the regulation of stem cell aging and hence, for tissue regeneration in the aging organism. Various different molecular mechanisms might be involved in this external control by the stem cell niche and future research will have to focus on this.

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References

1. Allsopp, R.C., Morin, G.B., DePinho, R., Harley, C.B., and Weissman, I.L., 2003a. Telomerase is required to slow telomere shortening and extend replicative lifespan of HSCs during serial transplantation. *Blood*, 102, 517-520.
2. Allsopp, R.C., Morin, G.B., Horner, J.W., DePinho, R., Harley, C.B., and Weissman, I.L., 2003b. Effect of TERT over-expression on the long-term transplantation capacity of hematopoietic stem cells. *Nat. Med.*, 9, 369-371.
3. Bartel, D.P., 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*, 116, 281-297.
4. Boehm, M. and Slack, F., 2005. A developmental timing microRNA and its target regulate life span in *C. elegans*. *Science*, 310, 1954-1957.
5. Bug, G., Gul, H., Schwarz, K., Pfeifer, H., Kampfmann, M., Zheng, X., Beissert, T., Boehrer, S., Hoelzer, D., Ottmann, O.G., and Ruthardt, M., 2005. Valproic acid stimulates proliferation and self-renewal of hematopoietic stem cells. *Cancer Res.*, 65, 2537-2541.
6. Chambers, S.M., Shaw, C.A., Gatz, C., Fisk, C.J., Donehower, L.A., and Goodell, M.A., 2007. Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation. *PLoS Biol.*, 5, 201.
7. Chen, J., 2004. Senescence and functional failure in hematopoietic stem cells. *Exp. Hematol.*, 32, 1025-1032.
8. Cheshier, S.H., Morrison, S.J., Liao, X., and Weissman, I.L., 1999. In vivo proliferation and cell cycle kinetics of long-term self-renewing hematopoietic stem cells. *Proc. Natl. Acad. Sci. U. S. A.*, 96, 3120-3125.
9. de Haan, G., Nijhof, W., and Van Zant, G., 1997. Mouse strain-dependent changes in frequency and proliferation of hematopoietic stem cells during aging: correlation between lifespan and cycling activity. *Blood*, 89, 1543-1550.
10. de Haan, G. and Van Zant, G., 1999. Dynamic changes in mouse hematopoietic stem cell numbers during aging. *Blood*, 93, 3294-3301.
11. de Wynter, E. and Ploemacher, R.E., 2001. Assays for the assessment of human hematopoietic stem cells. *J. Biol. Regul. Homeost. Agents*, 15, 23-27.
12. Dexter, T.M., Allen, T.D., Lajtha, L.G., Schofield, R., and Lord, B.I., 1973. Stimulation of differentiation and proliferation of haemopoietic cells in vitro. *J. Cell Physiol*, 82, 461-473.
13. Di Donna, S., Mamchaoui, K., Cooper, R.N., Seigneurin-Venin, S., Tremblay, J., Butler-Browne, G.S., and Mouly, V., 2003. Telomerase can extend the proliferative capacity of human myoblasts, but does not lead to their immortalization. *Mol. Cancer Res.*, 1, 643-653.
14. Dingli, D. and Pacheco, J.M., 2006. Allometric scaling of the active hematopoietic stem cell pool across mammals. *PLoS ONE.*, 1, e2.

15. Donnini, A., Re, F., Orlando, F., and Provinciali, M., 2007. Intrinsic and microenvironmental defects are involved in the age-related changes of Lin - c-kit+ hematopoietic progenitor cells. *Rejuvenation. Res.*, 10, 459-472.
16. Dykstra, B. and de Haan, G., 2008. Hematopoietic stem cell aging and self-renewal. *Cell Tissue Res.*, 331, 91-101.
17. Fehrer, C., Brunauer, R., Laschober, G., Unterluggauer, H., Reitinger, S., Kloss, F., Gully, C., Gassner, R., and Lepperdinger, G., 2007. Reduced oxygen tension attenuates differentiation capacity of human mesenchymal stem cells and prolongs their lifespan. *Aging Cell*, 6, 745-757.
18. Finkel, T. and Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. *Nature*, 408, 239-247.
19. Geiger, H., Koehler, A., and Gunzer, M., 2007. Stem cells, aging, niche, adhesion and Cdc42: a model for changes in cell-cell interactions and hematopoietic stem cell aging. *Cell Cycle*, 6, 884-887.
20. Giebel, B., Zhang, T., Beckmann, J., Spanholtz, J., Wernet, P., Ho, A.D., and Punzel, M., 2006. Primitive human hematopoietic cells give rise to differentially specified daughter cells upon their initial cell division. *Blood*, 107, 2146-2152.
21. Harrison, D.E. and Astle, C.M., 1982. Loss of stem cell repopulating ability upon transplantation. Effects of donor age, cell number, and transplantation procedure. *J. Exp. Med.*, 156, 1767-1779.
22. Hayflick, L., 2007. Biological aging is no longer an unsolved problem. *Ann. N. Y. Acad. Sci.*, 1100, 1-13.
23. Ho, A.D. and Wagner, W., 2007. The beauty of asymmetry – asymmetric divisions and self-renewal in the hematopoietic system. *Current Opinion in Hematology*, 14, 330-336.
24. Ho, A.D., Wagner, W., and Mahlknecht, U., 2005. Stem cells and ageing. The potential of stem cells to overcome age-related deteriorations of the body in regenerative medicine. *EMBO Rep.*, 6, 35-38.
25. Ibanez-Ventoso, C., Yang, M., Guo, S., Robins, H., Padgett, R.W., and Driscoll, M., 2006. Modulated microRNA expression during adult lifespan in *Caenorhabditis elegans*. *Aging Cell*, 5, 235-246.
26. Jones, D.L. and Wagers, A.J., 2008. No place like home: anatomy and function of the stem cell niche. *Nat. Rev Mol. Cell Biol.*, 9, 11-21.
27. Ju, Z., Jiang, H., Jaworski, M., Rathinam, C., Gompf, A., Klein, C., Trumpp, A., and Rudolph, K.L., 2007. Telomere dysfunction induces environmental alterations limiting hematopoietic stem cell function and engraftment. *Nat. Med.*, 13, 742-747.
28. Justesen, J., Stenderup, K., Ebbesen, E.N., Mosekilde, L., Steiniche, T., and Kassem, M., 2001. Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis. *Biogerontology.*, 2, 165-171.
29. Kenyon, J. and Gerson, S.L., 2007. The role of DNA damage repair in aging of adult stem cells. *Nucleic Acids Res.*, 35, 7557-7565.

30. Kiel, M.J., Yilmaz, O.H., Iwashita, T., Yilmaz, O.H., Terhorst, C., and Morrison, S.J., 2005. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell*, 121, 1109-1121.
31. Kim, M., Moon, H.B., and Spangrude, G.J., 2003. Major age-related changes of mouse hematopoietic stem/progenitor cells. *Ann. N. Y. Acad. Sci.*, 996, 195-208.
32. Kiyono, T., Foster, S.A., Koop, J.I., McDougall, J.K., Galloway, D.A., and Klingelutz, A.J., 1998. Both Rb/p16INK4a inactivation and telomerase activity are required to immortalize human epithelial cells. *Nature*, 396, 84-88.
33. Li, F., Jin, F., Freitas, A., Szabo, P., and Weksler, M.E., 2001. Impaired regeneration of the peripheral B cell repertoire from bone marrow following lymphopenia in old mice. *Eur. J. Immunol.*, 31, 500-505.
34. Liang, Y., Van Zant, G., and Szilvassy, S.J., 2005. Effects of aging on the homing and engraftment of murine hematopoietic stem and progenitor cells. *Blood*, 106, 1479-1487.
35. Linton, P.J. and Dorshkind, K., 2004. Age-related changes in lymphocyte development and function. *Nat. Immunol.*, 5, 133-139.
36. Mauch, P., Botnick, L.E., Hannon, E.C., Obbagy, J., and Hellman, S., 1982. Decline in bone marrow proliferative capacity as a function of age. *Blood*, 60, 245-252.
37. Morrison, S.J., Wandycz, A.M., Akashi, K., Globerson, A., and Weissman, I.L., 1996. The aging of hematopoietic stem cells. *Nat. Med.*, 2, 1011-1016.
38. O'Hare, M.J., Bond, J., Clarke, C., Takeuchi, Y., Atherton, A.J., Berry, C., Moody, J., Silver, A.R., Davies, D.C., Alsop, A.E., Neville, A.M., and Jat, P.S., 2001. Conditional immortalization of freshly isolated human mammary fibroblasts and endothelial cells. *Proc. Natl. Acad. Sci. U. S. A.*, 98, 646-651.
39. Parmar, K., Mauch, P., Vergilio, J.A., Sackstein, R., and Down, J.D., 2007. Distribution of hematopoietic stem cells in the bone marrow according to regional hypoxia. *Proc. Natl. Acad. Sci. U. S. A.*, 104, 5431-5436.
40. Rossi, D.J., Bryder, D., Seita, J., Nussenzweig, A., Hoeijmakers, J., and Weissman, I.L., 2007a. Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. *Nature*, 447, 725-729.
41. Rossi, D.J., Bryder, D., Zahn, J.M., Ahlenius, H., Sonu, R., Wagers, A.J., and Weissman, I.L., 2005. Cell intrinsic alterations underlie hematopoietic stem cell aging. *Proc. Natl. Acad. Sci. U. S. A.*, 102, 9194-9199.
42. Rossi, D.J., Seita, J., Czechowicz, A., Bhattacharya, D., Bryder, D., and Weissman, I.L., 2007b. Hematopoietic stem cell quiescence attenuates DNA damage response and permits DNA damage accumulation during aging. *Cell Cycle*, 6, 2371-2376.
43. Ruau, D., Ensenat, W.R., Dinger, T.C., Vallabhapurapu, D.S., Rolletschek, A., Hacker, C., Hieronymus, T., Wobus, A.M., Muller, A.M., and Zenke, M., 2008. Pluripotency Associated Genes are Reactivated by Chromatin Modifying Agents in Neurosphere Cells. *Stem Cells*.
44. Schofield, R., 1978. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells*, 4, 7-25.

45. Siminovich, L., Culloch, E.A., and TILL, J.E., 1963. The distribution of colony-forming cells among spleen colonies. *J. Cell Physiol*, 62, 327-336.
46. Sugiyama, T., Kohara, H., Noda, M., and Nagasawa, T., 2006. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. *Immunity*, 25, 977-988.
47. Wagner, W., Ansorge, A., Wirkner, U., Eckstein, V., Schwager, C., Blake, J., Miesala, K., Selig, J., Saffrich, R., Ansorge, W., and Ho, A.D., 2004. Molecular evidence for stem cell function of the slow-dividing fraction among human hematopoietic progenitor cells by genome-wide analysis. *Blood*, 104, 675-686.
48. Wagner, W. and Ho, A.D., 2007. Mesenchymal stem cell preparations-comparing apples and oranges. *Stem Cell Rev*, 3, 239-248.
49. Wagner, W., Roderburg, C., Wein, F., Diehlmann, A., Frankhauser, M., Schubert, R., Eckstein, V., and Ho, A.D., 2007a. Molecular and Secretory Profiles of Human Mesenchymal Stromal Cells and their Abilities to Maintain Primitive Hematopoietic Progenitors. *Stem Cells*, 10, 2638-2657.
50. Wagner, W., Saffrich, R., Wirkner, U., Eckstein, V., Blake, J., Ansorge, A., Schwager, C., Wein, F., Miesala, K., Ansorge, W., and Ho, A.D., 2005. Hematopoietic progenitor cells and cellular microenvironment: behavioral and molecular changes upon interaction. *Stem Cells*, 23, 1180-1191.
51. Wagner, W., Wein, F., Roderburg, C., Saffrich, R., Faber, A., Krause, U., Schubert, M., Benes, V., Eckstein, V., Maul, H., and Ho, A.D., 2007b. Adhesion of hematopoietic progenitor cells to human mesenchymal stem cells as a model for cell-cell interaction. *Exp. Hematol.*, 35, 314-325.
52. Wilson, A. and Trumpp, A., 2006. Bone-marrow haematopoietic-stem-cell niches. *Nat. Rev. Immunol.*, 6, 93-106.
53. Xing, Z., Ryan, M.A., Daria, D., Nattamai, K.J., Van Zant, G., Wang, L., Zheng, Y., and Geiger, H., 2006. Increased hematopoietic stem cell mobilization in aged mice. *Blood*, 108, 2190-2197.
54. Young, J.C., Wu, S., Hansteen, G., Du, C., Sambucetti, L., Remiszewski, S., O'Farrell, A.M., Hill, B., Lavau, C., and Murray, L.J., 2004. Inhibitors of histone deacetylases promote hematopoietic stem cell self-renewal. *Cytotherapy*, 6, 328-336.
55. Zhang, J., Niu, C., Ye, L., Huang, H., He, X., Tong, W.G., Ross, J., Haug, J., Johnson, T., Feng, J.Q., Harris, S., Wiedemann, L.M., Mishina, Y., and Li, L., 2003. Identification of the haematopoietic stem cell niche and control of the niche size. *Nature*, 425, 836-841.
56. Zhu, X., Gui, J., Dohkan, J., Cheng, L., Barnes, P.F., and Su, D.M., 2007. Lymphohematopoietic progenitors do not have a synchronized defect with age-related thymic involution. *Aging Cell*, 6, 663-672.

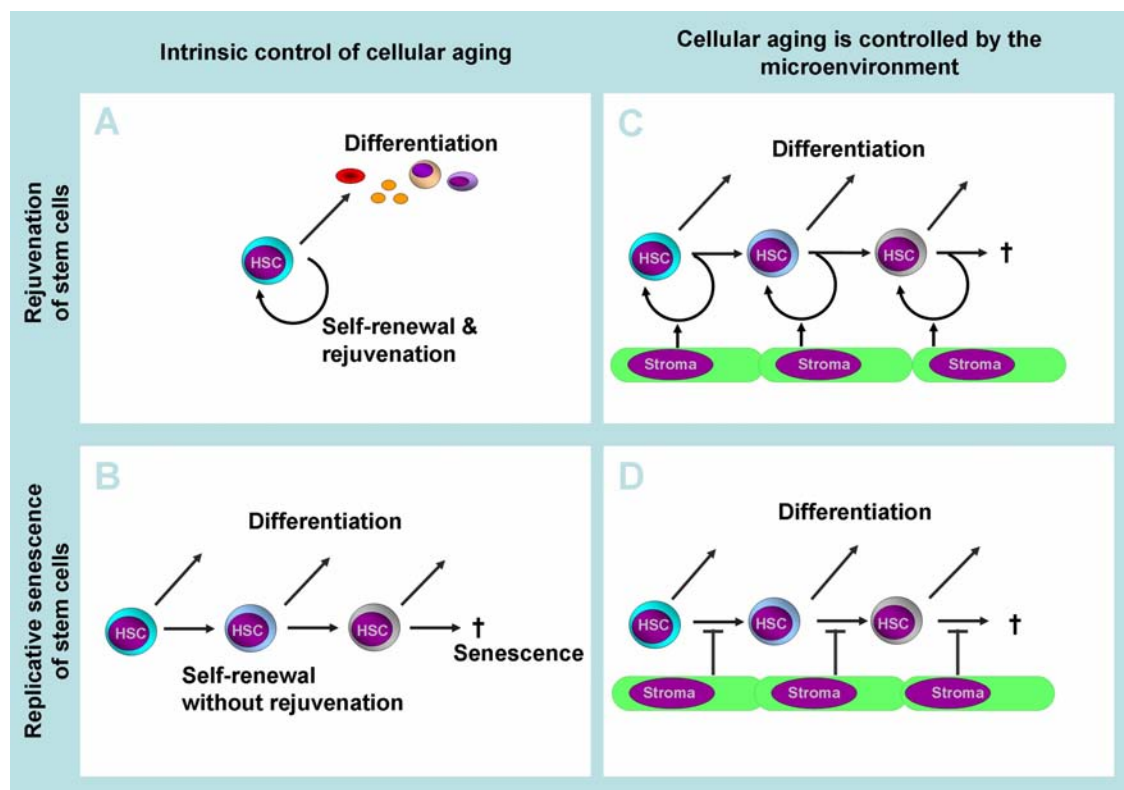


Fig 1. Potential mechanisms involved in aging of hematopoietic stem cells. Self-renewal of HSC might be associated with rejuvenation to circumvent cellular aging in adult stem cells (A). Alternatively HSC could be bound to replicative senescence with a limited number of cell divisions such as any other somatic cell (B). Aging of HSC might also be controlled by the external stimuli from the microenvironment that regulate rejuvenation of HSC (C). Last but not least, anchorage of HSC to specific niches in the bone marrow seems to keep them in a quiescent state and hence, interaction with the microenvironment might counteract replicative senescence of HSC (D).

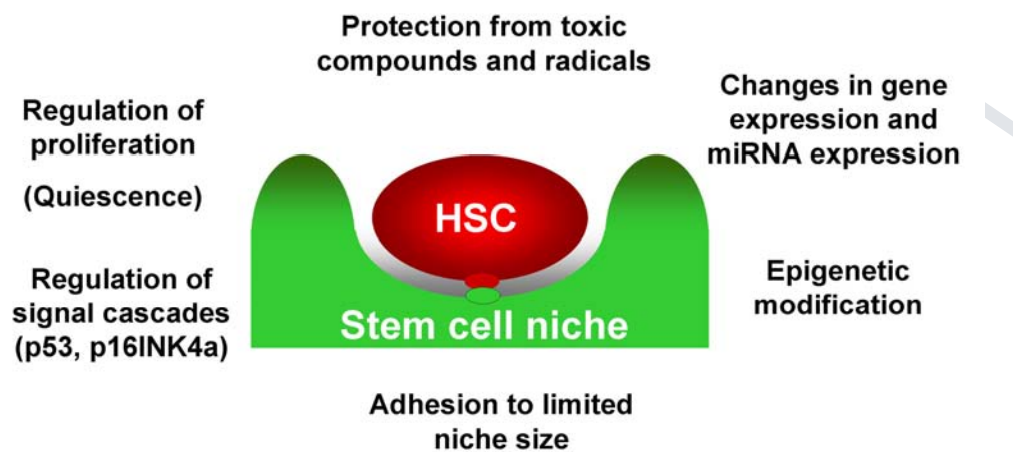


Fig 2. Potential mechanisms for regulation of aging by the stem cell niche. Self-renewal and differentiation of stem cells are tightly controlled by the stem cell niche in the bone marrow. This figure summarizes potential mechanisms by which this interaction might also regulate the process of aging in HSC.