STEM CELLS®

STEM CELL GENETICS AND GENOMICS: CONCISE REVIEW

Cellular Memory and Hematopoietic Stem Cell Aging

LEONIE M. KAMMINGA, GERALD DE HAAN

Department of Cell Biology, Section Stem Cell Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Key Words. Epigenetics • Chromatin • Aging • Cellular memory • Stem cells

ABSTRACT

Hematopoietic stem cells (HSCs) balance self-renewal and differentiation in order to sustain lifelong blood production and simultaneously maintain the HSC pool. However, there is clear evidence that HSCs are subject to quantitative and qualitative exhaustion. In this review, we briefly discuss several known aspects of the stem cell aging process, including DNA damage, telomere shortening, and oxidative stress. Besides these known players, there is increasing evidence that higher order chromatin structure, largely defined by the histone code and affecting transcriptional activity, is important. A model is suggested which describes how epigenetic regulation of gene transcription by modulation of the chromatin structure in stem cells can account for regulation of the aging program. STEM CELLS *2006;24:1143–1149*

STEM CELLS AND AGING

Embryonic and adult stem cells are capable of both self-renewal and differentiation. Through the poorly understood process of asymmetric partition of cellular constituents, a single cell division can result in the formation of both a newly formed stem cell and a more differentiated progenitor cell [1]. Differentiation often interfaces with proliferation, enabling a single stem cell to produce enormous numbers of fully differentiated, post-mitotic, tissue-specific end cells. For example, rare hematopoietic stem cells (HSCs) (only accounting for $<0.05\%$ of the total bone marrow [BM] cells) produce billions of blood cells each day in mammals [2–9]. All these differentiated cells have a limited life span. This life span may range from several hours (neutrophilic granulocytes, epithelial cells in the small intestines), many days to weeks (platelets, red blood cells, skin keratinocytes), to many years (lymphocyte subsets). The finite life span of somatic cells and their consequential loss are compensated by the production of new cells from stem cells. Evolutionarily more simple organisms, such as *Caenorhabditis elegans* and *Drosophila melanogaster*, are (almost) exclusively post-mitotic and are not believed to contain somatic stem cells. The life span of these species is largely accounted for by the collective life span of all (or most) of its individual cells. It is tempting to speculate that acquisition of adult stem cells during evolution has resulted in a major extension of organismal life span. Along these lines, it is reasonable to argue that the sole function of adult stem cells is to rejuvenate aged tissue.

Let us first define the process of aging as we will discuss it in this review. We propose that aging must be a continuous process that already starts in utero. After the second cell division of the zygote, commitment of both daughter cells to develop into certain lineages occurs. Already at this early stage, there is loss of cell potential. In the context of this work, we define aging as the gradual loss of cell potential. We will focus our discussion on the HSC system, because this model is best understood and amenable to experimental perturbation. Importantly, however, we believe that molecular mechanisms that specify HSC aging are likely operating in other cell systems.

HSCS AND AGING

HSCs reside in the BM and provide lifelong production of progenitors and peripheral blood cells. Simultaneously, HSCs must be able to maintain the stem cell pool by self-renewal divisions. In stem cell homeostasis, a delicate balance exists between self-renewal and terminal differentiation, because excessive self-renewal may initiate cancer (i.e., leukemia), and increased differentiation ultimately may lead to premature exhaustion of the stem cell pool. It is likely that during replicative stress (which can be experimentally induced by serial transplantation but may also result from normal aging) this balance weighs in favor of terminal differentiation, resulting in exhaustion of the HSC pool. An array of different assays are developed to assess stem cell potential, and multiple arrays should be used in order to claim true stem cell activity [10, 11]. Under normal conditions, the HSC pool is large enough to provide an organism with a sufficient number of committed progenitors to ensure homeostasis, even after serious bleeding or chemotherapy. Old people, or mice for that matter, typically do not die because they

Correspondence: Gerald de Haan, Ph.D., Department of Cell Biology, Section Stem Cell Biology, University Medical Center Groningen, University of Groningen, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands. Telephone: +31 50 3632722; Fax: +31 50 3637477; e-mail: g.de.haan@med.umcg.nl Received July 28, 2005; accepted for publication January 23, 2006. First published online in STEM CELLS *EXPRESS* February 2, 2006. 1066-5099/2006/$20.00/0 doi: 10.1634/stemcells.2005-0345

run out of HSCs. Similarly, it has been documented that HSCs can outlive their original donor upon repeated serial transplantation in lethally irradiated recipients [12]. Nevertheless, there is ample evidence that stem cell quality actually does decrease with each self-renewal division [13].

Many studies addressing the issue of HSC aging have used serial transplantations. Upon serial transplantation, HSCs undergo replicative stress and are in this way challenged to rescue lethally irradiated recipients, providing them with sufficient HSCs and multilineage reconstitution. It has been shown that serial transplantations can be carried out for only a restricted number of passages [12, 14–22]. In addition, functional decline of HSCs increased with repeated serial transfers [14, 15, 18, 20, 23, 24]. Demise of stem cell activity was observed when either purified HSCs [25, 26] or unfractionated BM cells were used [20, 21, 26]. It has been shown that also during normal hematopoiesis the stem cell compartment is subject to aging, because freshly isolated stem cells from young donors were able to engraft aged recipients and contribute to multilineage reconstitution [26]. Although this has been an area of much debate, the general consensus, based upon a large body of evidence, is that HSCs do deteriorate after replicative stress and during normal aging.

An issue that has frequently been addressed is whether stem cell aging is regulated by intrinsic or extrinsic factors. Stem cells are associated with stromal cells, which not only provide structural support and maintain the position of the stem cells, but also secrete various cytokines [27–31]. The profile of cytokine production changes during aging. Moreover, it has been suggested that early after transplantation the age of the microenvironment plays a more dominant role in determining the numbers of various lineages than does the age of the HSCs [32]. Recently, it has been shown that increasing the number of osteoblasts in the stem cell niche resulted in a parallel increase in the number of HSCs [33], indicating an important role for the microenvironment in controlling stem cell self-renewal and differentiation.

If stem cell aging were largely extrinsically regulated, one might contemplate studies searching for humoral factors that potentially could interfere in this process. However, evidence from mouse studies shows that the aging program is largely intrinsically regulated. To assess the genetic component regulating stem cell aging, HSC characteristics have been studied in different inbred mouse strains. HSCs of the commonly used C57BL/6 (B6) mice are hardly affected by aging, because stem cell numbers are increasing upon aging [34–36] and cells can outlive their original donor during serial transplantation [12]. However, homing properties of B6 HSCs are altered, reducing their ability to engraft recipients [32, 37]. In contrast, aged DBA/2 (D2) mice do show apparent exhaustion of the stem cell pool [34, 38, 39]. Interestingly, the maximum life span of different inbred mouse strains correlates negatively with the percentage of progenitors in the S-phase of the cell cycle. For example, progenitor cells from long-lived B6 mice have a relatively low cycling activity, whereas the stem cell pool increases with age and is relatively small. In contrast, D2 mice have a shorter life span than B6 mice, their progenitors show increased cycling activity, and their stem cell pool decreases upon aging and is relatively large [40]. This suggests that rapidly dividing cells exhaust faster. These genetic differences with respect to cycling activity and stem cell pool are still present when D2 and B6 cells co-exist in the same microenvironment [38, 39]. In addition, differences between the D2 and B6 stem cell compartment appear to be pre-determined and do not change over time [41]. This strongly suggests intrinsic regulation of the stem cell aging program. What cell-intrinsic mechanisms could possibly confer a form of cellular memory to stem cells?

Cellular Damage and Stem Cell Aging

Telomere Shortening

Telomeres, the structures protecting chromosome ends, have received much attention as a potential cell-intrinsic trigger to induce replicative senescence. The verdict as to what role telomeres may play during stem cell aging is still out. Telomere length is largely maintained by the enzyme telomerase. Whereas most somatic stem cells have telomerase activity, this is hardly detectable in differentiated cells [42]. During in vitro proliferation of human fibroblasts, but also during in vivo aging, a gradual shortening of the average length of telomeres is observed [43, 44]. Furthermore, it has been shown that telomere shortening occurs during serial transplantation of HSCs, coinciding with impaired functioning [45]. The best proof that telomere length is relevant in regulating cellular aging is derived from studies in which the consequences of telomerase deficiency were investigated. Telomerase-deficient HSCs showed reduced long-term repopulating capacity, concomitant with an increase in genetic instability [46]. In addition, normal murine HSCs can be serially transplanted four times but HSCs of telomerase deficient mice can be transplanted only twice [47]. Interestingly, humans suffering from the rare inherited disorder dyskeratosis congenita, which results from a mutation in the *hTR* gene, have reduced levels of telomerase activity and shortened telomeres. In these patients, BM failure is the principal cause of death [48, 49]. Interestingly, telomere shortening occurs rapidly in cell lines derived from patients who suffer from premature aging disorders, like Werner syndrome and ataxia telangiectasia [50].

A strong argument against a direct role for telomerase in preventing stem cell aging is the observation that HSCs from mTERT (murine telomerase reverse transcriptase) transgenic mice, in which telomerase is overexpressed and telomere length is preserved, cannot be serially transplanted more often than wild-type cells. This indicates that other mechanisms must be involved in regulating stem cell exhaustion [51].

DNA Damage

Each replication round of the genome during cell division results in numerous copy errors, but elaborate proofreading and editing mechanisms have evolved to correct these [52]. The appropriate cellular response after detection of DNA damage is an initial attempt at repair, but if damage is too extensive or compromises DNA metabolism, a signaling cascade triggers cellular senescence or death. The cumulative extent of DNA damage during the lifetime of a stem cell may potentially result in its demise. In several human disorders, such as Xeroderma Pigmentosum and Werner syndrome, inborn errors in the DNA repair machinery have dramatic clinical consequences, including tissue-specific cancer predisposition and/or segmental progeria [53]. It is unknown at present whether stem cells from these patients succumb prematurely to senescence.

Indications that DNA damage can actually result in HSC exhaustion originate from an example from recent studies published by Prasher et al. [54]. Mice deficient in Ercc1, a protein essential in nucleotide excision repair, were used to examine the effects of deficits in DNA repair on the hematopoietic system. *Ercc1* mutant mice have decreased responses to hematopoietic stress and showed exhaustion of hematopoietic progenitor activity, suggesting, but not proving, premature senescence of the HSC, as no classical HSC assays were performed.

DNA lesions can be induced by oxidative damage, resulting from free radical production. Numerous recent discoveries on both extension of life span as well as premature aging in model organisms from yeast to mice consistently support a connection among oxidative metabolism, stress resistance, and aging [53]. The aging process may therefore be influenced by energy restriction through a reduction in the metabolic "rate of living," ultimately leading to reduced oxidative damage. Increasing life span by prolonged caloric restriction has been demonstrated in yeast, worms, flies, fish, mice, and rats [55]. In addition, a large number of long-lived mutants that have been identified in *C. elegans* and *Drosophila* result from increased resistance to oxidative stress [56–58].

Interestingly, lifelong dietary restriction in mice resulted in increased HSC frequencies and improved HSC function, strongly suggesting a role for caloric restriction in delaying hematopoietic senescence and prevention of HSC aging [59]. Furthermore, studies in mice deficient in the "ataxia telangiectasia mutated" (*Atm*) gene showed that the self-renewal capacity of HSCs depends on Atm-mediated inhibition of oxidative stress. *Atm*-deficient mice showed progressive BM failure resulting from a defect in HSC function that was associated with elevated reactive oxygen species (ROS). Treatment with antioxidative agents restored the reconstitutive capacity of *Atm*-deficient HSCs and prevented BM failure [60]. The potential extrinsic modulation of a stem cell-intrinsic senescence program is an exciting new field of research warranting further study. An example of this, although in an entirely different system, was recently published. In these parabiotic experiments in which young and old mice shared a circulatory system, re-activation of old muscle and liver progenitor cells by an unknown extrinsic factor in young serum was shown [61].

Protein Damage

Proteins form the core of cellular functions as diverse as signal transduction, mitosis, transport systems, and chaperone activity, and as such it is conceivable that an age-related increase in oxidative protein damage could have important physiological consequences to an organism. Proteins can be modified by multiple reactions involving ROS. Among these reactions, carbonylation has attracted a great deal of attention due to its irreversible and irreparable nature. It appears that the classical enzymes involved in ROS detoxification (that is, superoxide dismutases, catalases, and peroxidases) are key members of the cellular defense against protein carbonylation [62]. Oxidative carbonylation has been identified as one important factor in protein function and removal [63–65]. A large number of studies have shown that protein carbonylation increases with age [66, 67]. Normally, carbonylated proteins are marked for proteolysis by the proteasome and specific proteases. However, carbonylated proteins are able to escape degradation and form high-molecular-weight aggregates that accumulate with age. Such carbonylated aggregates can become cytotoxic and have been associated with a large number of age-related disorders, including Alzheimer's disease, Parkinson's disease, and cancer [65, 68]. The asymmetrically dividing yeast *Saccharomyces cerevisiae* has evolved a Sir2p-dependent system that specifically retains carbonylated proteins in the mother cell compartment during mitotic cytokinesis [69]. Thus, the new progeny, in contrast to the mother cell, exhibits full reproductive potential and starts out with a markedly reduced load of damage [69]. In addition, recent studies on asymmetric division show involvement of these processes in the stem cell niche [1]. It will be of interest to clarify whether segregation of damaged proteins is a phenomenon that can also be observed in higher eukaryotes. Specifically, it would be interesting to assess its role during stem cell self-renewal or generation of the germ cell line.

Cellular Memory

During development of multicellular organisms, cells become different from one another by distinct use of their genetic program in response to transient stimuli, an example being lineage specification in hematopoiesis [70]. Long after such a stimulus has disappeared, cellular memory mechanisms still enable cells to "remember" their chosen fate over many cell divisions. This implies that to grow and maintain a specific, lineage-restricted state, particular configurations of gene expression need to be transmitted to daughter cells. Such heritable programs that do not involve mutations of the DNA are referred to as epigenetic alterations. The chromatin structure and its modifications play a fundamental role in establishment and maintenance of epigenetically controlled developmental decisions [71].

Nucleosomes, the fundamental structural units of chromatin, are comprised of the core histone octamer (H2A, H2B, H3, and H4) and the associated DNA that wraps around these eight histones. The precise organization of chromatin is critical for many cellular processes, including transcription, replication, repair, recombination, and chromosome segregation. Dynamic changes in chromatin structure are directly influenced by post-translational modifications of the amino-terminal tails of histones [72, 73]. The packaging of eukaryotic DNA into nucleosomal arrays presents a major obstacle to transcription that must be dealt with in order for the transcriptional machinery to access the DNA template. Gene expression is determined not only by the availability of combinations of transcription factors, but also by chromatin context. The discovery of enzyme complexes dedicated to chromatin remodeling has led to new insights into the mechanism of transcription [74–77]. Eukaryotic genomes are often described as transcriptionally active (euchromatin) or transcriptionally silent (heterochromatin) [78].

Two antagonizing groups of proteins, Polycomb (PcG) and trithorax (trxG), are required to maintain gene expression patterns of important developmental regulators during cellular proliferation. During development, TrxG proteins are transcriptional activators, whereas PcG proteins are transcriptional repressors, and both are very well conserved during evolution in different species. The PcG and TrxG proteins appear to form the molecular basis of cellular memory. The maintenance of cellular memory involves dynamic, regulated interactions between the

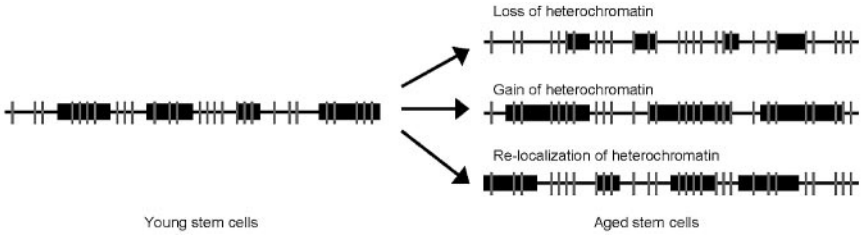

Figure 1. Possible mechanisms of change in heterochromatin structure during stem cell aging. Euchromatin is shown as thin black lines. Gradual changes in heterochromatin (black boxes) distribution occur during the transition from young to old stem cells. Because of these changes, distinct genes (gray bars) will be transcribed in young and old stem cells, leading to aberrant gene expression and potentially to the expression of non-stem cell genes. Stem cell aging may result from loss of heterochromatin, gain (spread) of heterochromatin, or re-localization of heterochromatin structures, all of which will result in perturbed gene expression profiles, impeding proper stem cell functioning.

PcG and TrxG proteins and their many target genes, via polycomb response elements (PREs) [71].

Is there any evidence that chromatin remodeling involving *PcG* or *TrxG* genes indeed confers memory to stem cells?

Cellular Memory and Stem Cells

There are two distinct PcG complexes (PcG repressive complex [PRC] 1 and 2) that associate with chromatin. Whereas PRC1 contains Cbx, Mph, Ring, Bmi1, and Mel18, PRC2 contains Ezh2, Eed, and Su(z)12 [79].

The role of selected PcG proteins in stem cell self-renewal has recently been established. *Mel-18* negatively regulates self-renewal of HSCs, because its loss leads to an increase of HSCs in G_0 and to enhanced HSC self-renewal [80]. *Mph1/Rae-28* mutant mice are embryonic lethal because HSC activity in these animals is not sufficient to maintain hematopoiesis during embryonic development [81]. HSCs derived from fetal liver isolated from *Bmi-1*$^{-/-}$ mice were not able to contribute to long-term hematopoiesis in competitive repopulation experiments, demonstrating cell-autonomous impairment of their self-renewal potential [82]. In contrast, overexpression of *Bmi-1* extends the replicative life span of mouse and human fibroblasts and causes lymphomas in transgenic mice [83]. Heterozygosity for a null allele of *Eed* caused marked myelo- and lymphoproliferative defects, indicating a negative regulation of cell cycle activity of both lymphoid and myeloid progenitor cells [84]. Overexpression of *Ezh2* in HSCs preserves stem cell potential and prevents HSC exhaustion after serial transplantation (L.M.K., G.d.H., paper resubmitted for publication). It is apparent from these data that PcG proteins are essential for normal HSC homeostasis.

TrxG proteins form complexes that are involved in general transcriptional processes, and therefore their function is not limited to epigenetic maintenance [85, 86]. Four complexes that contain TrxG proteins have been purified from *Drosophila* embryos, all with different chromatin-modifying properties [87]. The function of TrxG proteins with respect to HSC functioning has not been well studied. However, inappropriately expressed *TrxG* genes seem to be involved in tumor formation [88]. For example, the mixed lineage leukemia gene (*MLL1*), which was recently shown to be a histone 3 Lysine 4-specific methyltransferase [89, 90], is involved in 11q23 translocations in acute leukemias [91–93]. Studies with *Mll* mutant mice demonstrated an intrinsic requirement for *Mll* in definite hematopoiesis, in which it is essential for the generation of HSCs during embryogenesis [94].

Naturally occurring microRNAs (miRNAs) also constitute a powerful route to dynamically silence specific gene expression, and it is conceivable that such mechanisms may induce silencing initiation prior to the heterochromatization process that is mediated by histone methyltransferase-mediated lysine 9 and 27 histone H3 methylation [95]. It has been suggested that miRNAs might function in regulating development and therefore also play a role during cellular aging [96]. miRNAs that are specifically expressed in hematopoietic cells have been identified. These miRNAs presumably act by pairing to the mRNAs of their target genes to direct gene-silencing processes that are critical for hematopoiesis and possibly also for HSC self-renewal [97].

Cellular Memory and Aging of Stem Cells

Can we conceptually understand how "cellular memory" may contribute to aging? It has been suggested that during normal aging the structure of heterochromatin changes [98, 99]. Because heterochromatin domains must be epigenetically regenerated each time DNA is repaired or replicated, DNA damage and cell division are the major perturbing factors triggering heterochromatin loss. Loss of heterochromatin has therefore been implicated to reflect the number of cell divisions, or cycles of DNA damage and repair, resulting in multiple subtle changes in gene expression [99].

The heterochromatin island hypothesis postulates that repressive chromatin structures are scattered over the genome, reflecting the diverse genomic structure in individual cells within a tissue or among various tissues. For instance, even though brain cells and hematopoietic cells contain the same DNA sequences, due to transcriptional regulation these cells have their own specialized functions and specific characteristics. This model assumes that dynamic changes in the equilibrium in heterochromatin islands, rather than their simple unfolding or loss, are the essential driving force of cellular aging [98].

Others suggest a relatively open chromatin structure in stem cells, allowing many transcriptional options (a "promiscuous" beginning). Upon aging or differentiation, spread of heterochromatin can be expected, concomitant with a decrease of multilineage potential [100]. Using gene arrays, it has been shown (in keeping with this hypothesis) that in HSCs more distinct transcripts are present than in committed progenitors. In addition,

transcripts common to both HSCs and neural stem cells were identified, raising the possibility of extended commonality in the molecular ground states of HSCs and neural stem cells [101–103].

It is clear that epigenetic marks are set throughout embryogenesis and adult life and that this is an important mechanism to guide proper gene transcription. However, it remains uncertain to what extent heterochromatin structure changes during differentiation and aging. Even though epigenetic marks are relatively rigid and stable, we hypothesize that during stem cell aging, and concomitant with cell replication, the histone code in stem cells gradually is altered, ultimately resulting in impaired functioning. The different outcomes with respect to changes in heterochromatin structure during differentiation (i.e., loss of heterochromatin, gain of heterochromatin, and re-localization of heterochromatin [Fig. 1]) are probably also processes that are important during stem cell aging. Aging might result from a general loss of heterochromatin, as a consequence of which non-stem cell genes might be transcribed, thereby impeding proper stem cell functioning. A second model suggests that in stem cells many transcriptional options are available and that during cellular aging, as a consequence of heterochromatin spreading, these options are gradually shut down. A third model suggests mere re-localization of the heterochromatin structures, similarly resulting in stem cell-noncompatible gene expression profiles. Whether and how the heterochromatin structure will alter during stem cell aging will be a new field to explore. Data documenting the involvement of chromatin-modifying proteins in stem cell self-renewal provide an attractive platform to test these hypotheses. A recent paper has documented gene reprogramming after fusion of adult cells with embryonic stem cells [104]. We suggest that modulation of the methylation and acetylation patterns of chromatin by cellular, genetic, or pharmacological means may rejuvenate stem cells.

ACKNOWLEDGMENTS

This work was supported by grants form the Dutch Organization for Scientific Research (NOW; grant no. 901-08-339) and the National Institutes of Health (RO1 HL073710).

DISCLOSURES

The authors indicate no potential conflicts of interest.

REFERENCES

1 Ho AD. Kinetics and symmetry of divisions of hematopoietic stem cells. Exp Hematol 2005;33:1–8.

2 Chen J, Astle CM, Harrison DE. Development and aging of primitive hematopoietic stem cells in BALB/cBy mice. Exp Hematol 1999;27:928–935.

3 Harrison DE, Astle CM, Stone M. Numbers and functions of transplantable primitive immunohematopoietic stem cells. Effects of age. J Immunol 1989;142:3833–3840.

4 Zhao Y, Lin Y, Zhan Y et al. Murine hematopoietic stem cell characterization and its regulation in BM transplantation. Blood 2000;96:3016–3022.

5 Chen J, Astle CM, Muller-Sieburg CE et al. Primitive hematopoietic stem cell function in vivo is uniquely high in the CXB-12 mouse strain. Blood 2000;96:4124–4131.

6 Harrison DE, Jordan CT, Zhong RK et al. Primitive hemopoietic stem cells: Direct assay of most productive populations by competitive repopulation with simple binomial, correlation and covariance calculations. Exp Hematol 1993;21:206–219.

7 Muller-Sieburg CE, Riblet R. Genetic control of the frequency of hematopoietic stem cells in mice: Mapping of a candidate locus to chromosome 1. J Exp Med 1996;183:1141–1150.

8 Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. Science 1988;241:58–62.

9 Osawa M, Hanada K, Hamada H et al. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. Science 1996;273:242–245.

10 van Os R, Kamminga LM, de Haan G. Stem cell assays: Something old, something new, something borrowed. STEM CELLS 2004;22:1181–1190.

11 Coulombel L. Identification of hematopoietic stem/progenitor cells: Strength and drawbacks of functional assays. Oncogene 2004;23:7210–7222.

12 Harrison DE. Mouse erythropoietic stem cell lines function normally 100 months: Loss related to number of transplantations. Mech Ageing Dev 1979;9:427–433.

13 Van Zant G, de Haan G, Rich-Ivan N. Alternatives to stem cell renewal from a developmental viewpoint. Exp Hematol 1997;1997:187–192.

14 Siminovitch L, Till JE, McCullock EA. Decline in colony-forming ability of marrow cells subjected to serial transplantion into irradiated mice. J Cell Comp Physiol 1964;64:23.

15 Cudkowicz G, Upton AC, Shearer GM. Lymphocyte content and proliferative capacity of serially transplanted mouse bone marrow. Nature 1964;201:165.

16 Micklem HS, Ogden DA. Ageing of haematopoietic cell populations in the mouse. In: Cairnie AB, Lala PK, Osmond DG, eds. Stem Cells of Renewing Cell Populations. New York: Academic Press, Inc., 1976:331–341.

17 Ogden DA, Mickliem HS. The fate of serially transplanted bone marrow cell populations from young and old donors. Transplantation 1976;22:287–293.

18 Harrison DE, Astle CM, Delaittre JA. Loss of proliferative capacity in immunohemopoietic stem cells caused by serial transplantation rather than aging. J Exp Med 1978;147:1526–1531.

19 Hellman S, Botnick LE, Hannon EC et al. Proliferative capacity of murine hematopoietic stem cells. Proc Natl Acad Sci U S A 1978;75:490–494.

20 Ross EA, Anderson N, Micklem HS. Serial depletion and regeneration of the murine hematopoietic system. Implications for hematopoietic organization and the study of cellular aging. J Exp Med 1982;155:432–444.

21 Harrison DE, Astle CM. Loss of stem cell repopulating ability upon transplantation. Effects of donor age, cell number, and transplantation procedure. J Exp Med 1982;156:1767–1779.

22 Iscove NN, Nawa K. Hematopoietic stem cells expand during serial transplantation in vivo without apparent exhaustion. Curr Biol 1997;7:805–808.

23 Harrison DE, Stone M, Astle CM. Effects of transplantation on the primitive immunohematopoietic stem cell. J Exp Med 1990;172:431–437.

24 Micklem HS, Ross E. Heterogeneity and ageing of haematopoietic stem cells. Ann Immunol (Paris) 1978;129:367–376.

25 Spangrude GJ, Brooks DM, Tumas DB. Long-term repopulation of irradiated mice with limiting numbers of purified hematopoietic stem cells: In vivo expansion of stem cell phenotype but not function. Blood 1995;85:1006–1016.

26 Kamminga LM, van Os R, Ausema A et al. Impaired hematopoietic stem cell functioning after serial transplantation and during normal aging. STEM CELLS 2005;23:82–92.

27 Gordon MY, Lewis JL, Marley SB et al. Stromal cells negatively regulate primitive haemopoietic progenitor cell activation via a phosphatidylinositol-anchored cell adhesion/signalling mechanism. Br J Haematol 1997;96:647–653.

28 Hackney JA, Charbord P, Brunk BP et al. A molecular profile of a hematopoietic stem cell niche. Proc Natl Acad Sci U S A 2002;99:13061–13066.

29 Walker JA, Lunch M, Silverman S et al. The Notch/Jagged pathway inhibits proliferation of human hematopoietic progenitors in vitro. STEM CELLS 1999;17:162–171.

30 Wilson JG. Adhesive interactions in hemopoiesis. Acta Haematologica 1997;97:6–12.

31 Yoder MC, Williams DA. Matrix molecule interactions with hematopoietic stem cells. Exp Hematol 1995;23:961–967.

32 Liang Y, Van Zant G, Szilvassy SJ. Effects of aging on the homing and engraftment of murine hematopoietic stem and progenitor cells. Blood 2005;106:1479–1487.

33 Calvi LM, Adams GB, Weibrecht KW et al. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature 2003;425:841–846.

34 Chen J, Astle CM, Harrison DE. Genetic regulation of primitive hematopoietic stem cell senescence. Exp Hematol 2000;28:442–450.

35 de Haan G, Van Zant G. Dynamic changes in mouse hematopoietic stem cell numbers during aging. Blood 1999;93:3294–3301.

36 Sudo K, Ema H, Morita Y et al. Age-associated characteristics of murine hematopoietic stem cells. J Exp Med 2000;192:1273–1280.

37 Morrison SJ, Wandycz AM, Akashi K et al. The aging of hematopoietic stem cells. Nat Med 1996;2:1011–1016.

38 Kamminga LM, Akkerman I, Weersing E et al. Autonomous behavior of hematopoietic stem cells. Exp Hematol 2000;28:1451–1459.

39 Van Zant G, Holland BP, Eldridge PW et al. Genotype-restricted growth and aging patterns in hematopoietic stem cell populations of allophenic mice. J Exp Med 1990;171:1547–1565.

40 de Haan G, Nijhof W, Van Zant G. Mouse strain-dependent changes in frequency and proliferation of hematopoietic stem cells during aging: Correlation between lifespan and cycling activity. Blood 1997;89:1543–1550.

41 Roeder I, Kamminga LM, Braesel K et al. Competitive clonal hematopoiesis in mouse chimeras explained by a stochastic model of stem cell organization. Blood 2005;105:609–616.

42 Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell 1985;43:405–413.

43 Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature 1990;345:458–460.

44 Vaziri H, Dragowska W, Allsopp RC et al. Evidence for a mitotic clock in human hematopoietic stem cells: Loss of telomeric DNA with age. Proc Natl Acad Sci U S A 1994;91:9857–9860.

45 Allsopp RC, Cheshier S, Weissman IL. Telomere shortening accompanies increased cell cycle activity during serial transplantation of hematopoietic stem cells. J Exp Med 2001;193:917–924.

46 Samper E, Fernandez P, Eguia R et al. Long-term repopulating ability of telomerase-deficient murine hematopoietic stem cells. Blood 2002;99:2767–2775.

47 Allsopp RC, Morin GB, DePinho R et al. Telomerase is required to slow telomere shortening and extend replicative lifespan of HSCs during serial transplantation. Blood 2003;102:517–520.

48 Marrone A, Mason PJ. Dyskeratosis congenita. Cell Mol Life Sci 2003;60:507–517.

49 Mason PJ. Stem cells, telomerase and dyskeratosis congenita. Bioessays 2003;25:126–133.

50 Hande MP. DNA repair factors and telomere-chromosome integrity in mammalian cells. Cytogenet Genome Res 2004;104:116–122.

51 Allsopp RC, Morin GB, Horner JW et al. Effect of TERT overexpression on the long-term transplantation capacity of hematopoietic stem cells. Nat Med 2003;9:369–371.

52 Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. Nature 2001;411:366–374.

53 Mitchell JR, Hoeijmakers JH, Niedernhofer LJ. Divide and conquer: Nucleotide excision repair battles cancer and ageing. Curr Opin Cell Biol 2003;15:232–240.

54 Prasher JM, Lalai AS, Heijmans-Antonissen C et al. Reduced hematopoietic reserves in DNA interstrand crosslink repair-deficient Ercc1−/− mice. EMBO J 2005;24:861–871.

55 Smith JV, Heilbronn LK, Ravussin E. Energy restriction and aging. Curr Opin Clin Nutr Metab Care 2004;7:615–622.

56 Van Zant G, Liang Y. The role of stem cells in aging. Exp Hematol 2003;31:659–672.

57 Bokov A, Chaudhuri A, Richardson A. The role of oxidative damage and stress in aging. Mech Ageing Dev 2004;125:811–826.

58 Harper ME, Bevilacqua L, Hagopian K et al. Ageing, oxidative stress, and mitochondrial uncoupling. Acta Physiol Scand 2004;182:321–331.

59 Chen J, Astle CM, Harrison DE. Hematopoietic senescence is postponed and hematopoietic stem cell function is enhanced by dietary restriction. Exp Hematol 2003;31:1097–1103.

60 Ito K, Hirao A, Arai F et al. Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. Nature 2004;431:997–1002.

61 Conboy IM, Conboy MJ, Wagers AJ et al. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature 2005;433:760–764.

62 Nystrom T. Role of oxidative carbonylation in protein quality control and senescence. EMBO J 2005;24:1311–1317.

63 Oliver CN, Ahn BW, Moerman EJ et al. Age-related changes in oxidized proteins. J Biol Chem 1987;262:5488–5491.

64 Stadtman ER. Protein oxidation and aging. Science 1992;257:1220–1224.

65 Levine RL. Carbonyl modified proteins in cellular regulation, aging, and disease. Free Radic Biol Med 2002;32:790–796.

66 Yan LJ, Sohal RS. Mitochondrial adenine nucleotide translocase is modified oxidatively during aging. Proc Natl Acad Sci U S A 1998;95:12896–12901.

67 Yan LJ, Levine RL, Sohal RS. Oxidative damage during aging targets mitochondrial aconitase. Proc Natl Acad Sci U S A 1997;94:11168–11172.

68 Dalle-Donne I, Giustarini D, Colombo R et al. Protein carbonylation in human diseases. Trends Mol Med 2003;9:169–176.

69 Aguilaniu H, Gustafsson L, Rigoulet M et al. Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. Science 2003;299:1751–1753.

70 Tagoh H, Melnik S, Lefevre P et al. Dynamic reorganization of chromatin structure and selective DNA demethylation prior to stable enhancer complex formation during differentiation of primary hematopoietic cells in vitro. Blood 2004;103:2950–2955.

71 Ringrose L, Paro R. Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. Annu Rev Genet 2004;38:413–443.

72 Wolffe AP, Hayes JJ. Chromatin disruption and modification. Nucleic Acids Res 1999;27:711–720.

73 Luger K, Richmond TJ. The histone tails of the nucleosome. Curr Opin Genet Dev 1998;8:140–146.

74 Wolffe AP, Guschin D. Review: Chromatin structural features and targets that regulate transcription. J Struct Biol 2000;129:102–122.

75 Wu J, Grunstein M. 25 years after the nucleosome model: Chromatin modifications. Trends Biochem Sci 2000;25:619–623.

76 Workman JL, Kingston RE. Alteration of nucleosome structure as a mechanism of transcriptional regulation. Annu Rev Biochem 1998;67:545–579.

77 Kornberg RD, Lorch Y. Chromatin-modifying and -remodeling complexes. Curr Opin Genet Dev 1999;9:148–151.

78 Rice JC, Allis CD. Histone methylation versus histone acetylation: New insights into epigenetic regulation. Curr Opin Cell Biol 2001;13:263–273.

79 Lund AH, van Lohuizen M. Polycomb complexes and silencing mechanisms. Curr Opin Cell Biol 2004;16:239–246.

80 Kajiume T, Ninomiya Y, Ishihara H et al. Polycomb group gene mel-18 modulates the self-renewal activity and cell cycle status of hematopoietic stem cells. Exp Hematol 2004;32:571–578.

81 Ohta H, Sawada A, Kim JY et al. Polycomb group gene rae28 is required for sustaining activity of hematopoietic stem cells. J Exp Med 2002;195:759–770.

82 Park IK, Qian D, Kiel M et al. Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. Nature 2003;423:302–305.

83 Jacobs JJ, Kieboom K, Marino S et al. The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus. Nature 1999;397:164–168.

84 Lessard J, Schumacher A, Thorsteinsdottir U et al. Functional antagonism of the Polycomb-Group genes eed and Bmi1 in hemopoietic cell proliferation. Genes Dev 1999;13:2691–2703.

85 Collins RT, Furukawa T, Tanese N et al. Osa associates with the Brahma chromatin remodeling complex and promotes the activation of some target genes. EMBO J 1999;18:7029–7040.

86 Smith ST, Petruk S, Sedkov Y et al. Modulation of heat shock gene expression by the TAC1 chromatin-modifying complex. Nat Cell Biol 2004;6:162–167.

87 Simon JA, Tamkun JW. Programming off and on states in chromatin: Mechanisms of Polycomb and trithorax group complexes. Curr Opin Genet Dev 2002;12:210–218.

88 Lund AH, van Lohuizen M. Epigenetics and cancer. Genes Dev 2004; 18:2315–2335.

89 Milne TA, Briggs SD, Brock HW et al. MLL targets SET domain methyltransferase activity to Hox gene promoters. Mol Cell 2002;10: 1107–1117.

90 Nakamura T, Mori T, Tada S et al. ALL-1 is a histone methyltransferase that assembles a supercomplex of proteins involved in transcriptional regulation. Mol Cell 2002;10:1119–1128.

91 Djabali M, Selleri L, Parry P et al. A trithorax-like gene is interrupted by chromosome 11q23 translocations in acute leukaemias. Nat Genet 1992;2:113–118.

92 Gu Y, Nakamura T, Alder H et al. The t(4;11) chromosome translocation of human acute leukemias fuses the ALL-1 gene, related to Drosophila trithorax, to the AF-4 gene. Cell 1992;71:701–708.

93 Tkachuk DC, Kohler S, Cleary ML. Involvement of a homolog of Drosophila trithorax by 11q23 chromosomal translocations in acute leukemias. Cell 1992;71:691–700.

94 Ernst P, Fisher JK, Avery W et al. Definitive hematopoiesis requires the mixed-lineage leukemia gene. Dev Cell 2004;6:437–443.

95 Verdel A, Jia S, Gerber S et al. RNAi-mediated targeting of heterochromatin by the RITS complex. Science 2004;303:672–676.

96 Pasquinelli AE, Hunter S, Bracht J. MicroRNAs: A developing story. Curr Opin Genet Dev 2005;15:200–205.

97 Chen CZ, Li L, Lodish HF et al. MicroRNAs modulate hematopoietic lineage differentiation. Science 2004;303:83–86.

98 Imai S, Kitano H. Heterochromatin islands and their dynamic reorganization: A hypothesis for three distinctive features of cellular aging. Exp Gerontol 1998;33:555–570.

99 Villeponteau B. The heterochromatin loss model of aging. Exp Gerontol 1997;32:383–394.

100 Enver T, Greaves M. Loops, lineage, and leukemia. Cell 1998;94:9–12.

101 Terskikh AV, Miyamoto T, Chang C et al. Gene expression analysis of purified hematopoietic stem cells and committed progenitors. Blood 2003;102:94–101.

102 Akashi K, He X, Chen J et al. Transcriptional accessibility for genes of multiple tissues and hematopoietic lineages is hierarchically controlled during early hematopoiesis. Blood 2003;101:383–389.

103 Rossi DJ, Bryder D, Zahn JM et al. Cell intrinsic alterations underlie hematopoietic stem cell aging. Proc Natl Acad Sci U S A 2005;102: 9194–9199.

104 Cowan CA, Atienza J, Melton DA et al. Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. Science 2005;309:1369–1373.