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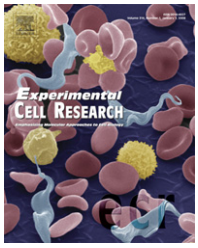

Review

Self-renewal and differentiation capacity of young and aged stem cells

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ABSTRACT

Because of their ability to self-renew and differentiate, adult stem cells are the *in vivo* source for replacing cells lost on a daily basis in high turnover tissues during the life of an organism. Adult stem cells however, do suffer the effects of aging resulting in decreased ability to self-renew and properly differentiate. Aging is a complex process and identification of the mechanisms underlying the aging of (stem) cell population(s) requires that relatively homogenous and well characterized populations can be isolated. Evaluation of the effect of aging on one such adult stem cell population, namely the hematopoietic stem cell (HSC), which can be purified to near homogeneity, has demonstrate that they do suffer cell intrinsic age associated changes. The cells that support HSC, namely marrow stromal cells, or mesenchymal stem cells (MSC), may similarly be affected by aging, although the inability to purify these cells to homogeneity precludes definitive assessment. As HSC and MSC are being used in cell-based therapies clinically, improved insight in the effect of aging on these two stem cell populations will probably impact the selection of sources for these stem cells.

Contents

Embryonic and adult stem cells

Stem cells can be defined by 3 main criteria: (1) ability to self-renew, for several cell divisions, which is a prerequisite for sustaining the stem cell pool, (2) ability to generate at the single cell level differentiated progeny cells, in general of multiple lineages and (3) the ability to functionally reconstitute a given tissue *in vivo* [1]. Cells derived from many different

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sources have been shown to fulfill those criteria, including embryonic stem cells (ESCs) and adult stem cells isolated from various tissues.

The typical adult stem cell, present in many tissues, is considered multipotent as it can only give rise to differentiated cell types from the tissue of origin. For example, the hematopoietic stem cell (HSC) can undergo self-renewing cell divisions *in vivo*, differentiate at the single cell level into all mature blood cells and functionally repopulate the hematopoietic system of a myeloablated recipient. A number of other adult stem cells have been identified, although they are not as well characterized as the HSC. Neural stem cells (NSCs) give rise to neurons, astrocytes and oligodendrocytes, the three main types of nerve cells in the adult brain [2]. Mesenchymal stem cells (MSC) differentiate into fibroblasts, adipocytes, osteoblasts, chondrocytes and skeletal muscle cells [3]. Other stem cells like spermatogonial, corneal and endothelial stem cells fulfill also the criteria of a stem cell, except that they differentiate only into a single type of differentiated cell. The 'multipotency' of adult stem cells is in contrast with the 'pluripotency' of ESCs, which can give rise to cells from the three somatic germ layers (ectoderm, mesoderm and endoderm) as well as to germ cells [4,5].

Most, if not all, human adult cells can undergo at most 50–60 divisions before they become senescent. This limited replicative capacity is also known as the 'Hayflick limit', according to its discoverer Leonard Hayflick [6] and it is thought to be determined by chromosomal telomere shortening after each cell division. It is well known that cells can escape this process *in vitro* by acquiring mutations in specific genes in a process called transformation. In contrast, ESCs possess a very high degree of self-renewal ability without requiring transformations to acquire these properties, at least in part due to the fact that they express, in contrast to adult cells, high levels of telomerase [7]. Nevertheless, both mouse and human ESCs acquire genetic abnormalities when cultured long-term, and perhaps not surprisingly, some of these abnormalities reinforce the self-renewal ability, and may even lock ESC in a state of self-renewal with loss of differentiation ability [8].

Pluripotent ESCs are derived from pluripotent cells in the inner cell mass of the blastocyst. *In vivo*, cells in the blastocyst become lineage restricted at day 4 of mouse (except in diapause) and day 7–10 of human development when they commit to a germline or somatic cell fate and subsequently to more tissue specific cells [9]. The characteristic of ESC, namely their theoretical infinite self-renewal capacity has to be maintained *in vitro* by specific culture conditions that allow them to self-renew and prevent them from differentiating. Remarkably; when these *in vitro* expanded ESCs are injected into blastocysts, they are able to resume a normal developmental program and contribute to tissues of all germ layers [10]. A recent series of studies has shown that the properties of infinite self-renewal ability and pluripotency (including chimeric somatic and germ line contribution) can be conferred to differentiated cells by introduction of three or four transcription factors [11–13]. Whether such de-differentiation is more easily accomplished when ontogenically less mature cells are used, or when adult stem cells vs. more differentiated adult cells are used, is not yet clear. Park et al, however, demonstrated that fibroblasts derived from human ESCs or from human fetus can be reprogrammed to an induced pluripotent (iPS) cell using Oct4, Sox2, Klf4 and cMyc, whereas the efficiency of reprogramming of neonatal and adult derived fibroblasts as well as adult derived MSC was significantly lower, and required in addition transduction with telomerase (hTERT) and SV40 large T antigen [14]. Whether this indicates that once cells have started to undergo telomere shortening upon multiple cell divisions during the life of the organism followed by loss of telomere length *in vitro* upon culture, the process of re-acquiring extensive self-renewal ability is jeopardized will need to be determined.

Adult stem cells on the other hand are able to self-renew to some extent *in vivo* which is required for maintaining tissue homeostasis in tissues where very large numbers of differentiated progeny need to be generated on a daily base, such as in the hematopoietic system, the epithelium of skin and gut. In other tissues where differentiated progeny does not turnover quickly such as the CNS, or where proliferation of terminally differentiated cells is in part responsible for maintaining tissue homeostasis such as in the liver, tissue specific stem cells serve as a reserve for cellular replacement or repair in case of minor or major injury, respectively [15]. In many mammalian tissues there is a decline in the ability to replace mature cells with age. It is reasonable to hypothesize that some of this decline could be related to decreased stem cell functionality with age, resulting in reduced ability to produce progenitors and differentiated cells that ultimately compromise the tissue or organ. It is still not clear whether this is due to the intrinsic aging of the cells, due to changes in the niche or to the external environment. The relative contribution of these factors might be different in different organs or tissues. However, the thesis that accumulation of senescent cells contributes to an 'aging' phenotype *in vivo* is still valid. The process of senescence likely represents a means to eliminate cells that acquired DNA damage and/or became transformed, that compromise organismal integrity (reviewed by [16]).

In this review we will focus on the self-renewal and differentiation capacity of young and aging stem cells from the perspective of tissue and organismal age, specifically hematopoietic stem cells and mesenchymal stem cells.

Aging of hematopoietic stem cells (HSC)

Hematopoietic stem cells (HSC) are responsible for producing all mature blood cells, throughout an organism's life. Even though, the hematopoietic system produces many millions of cells on a daily basis, the most primitive HSC, also termed the long-term repopulating HSC (LTR-HSC) is a relatively quiescent cell during steady state hematopoiesis. The daily replenishment of blood cells is achieved in large part by divisions and subsequent stepwise differentiation of cells that are descendants of the most primitive HSC, namely short term repopulating HSC (STR-HSC) and slightly more committed hematopoietic progenitor cells (HPC). The relative quiescence of HSC protects their genomic integrity by reducing the rounds of DNA replication and thus the probability of acquiring DNA damage that might compromise multilineage differentiation potential and/or render them malignant over time. Nevertheless, despite the fact that LTR-HSCs are in general quiescent, they appear to age with the host.

Murine HSC can now be purified to near homogeneity through the use of cell surface markers and can be evaluated using quantitative functional assays. This has allowed investigators to study the effect of aging on the functional behavior of HSC *in vivo*. Already in the 70's, Harrison et al found that serial transplantation of murine HSC is possible, demonstrating their ability to self-renew through multiple generations, outliving the donor mouse [17]. However, even in laboratory mice endowed with very long telomeres, multiple rounds of transplantation lead to telomere shortening [18]. There is also evidence that the human hematopoietic system ages, as telomere length of hematopoietic cells decreases with age. Hence, although HSC express telomerase, the level – which is substantially lower than in ESC – is insufficient to prevent progressive telomere shortening. This is also illustrated by the observation that upon transplantation, telomere length in blood cells of the recipient are 1–2 kb shorter than those in the donor, when evaluated several years following transplantation [19]. In a number of patients undergoing autologous HSC transplantation following extensive treatment with conventional chemotherapy, development of a myelodysplastic syndrome (MDS) has been documented [20]. The chemotherapy exposure prior to transplantation may have caused genetic mutations; an alternative explanation is that the many rounds of self-renewal of the HSC compartment prior to autografting may have lead to progressive telomere shortening and acquisition of genetic abnormalities possibly as a means to escape senescence.

Patients with dyskeratosis congenita (DKC), an inherited disease with mutations in genes that encode for the telomerase complex, present clinically with an impaired proliferative capacity of cells within high turnover tissues including skin and the hematopoietic system. In 80% of DKC patients, this leads to the development of bone marrow (BM) failure [21,22]. Circulating leukocytes of DKC patients have shorter telomeres, a feature also seen in patients with Fanconi anemia (FA). Aside from increased apoptosis, hematopoietic cells from FA-patients also have accelerated telomere shortening rates *in vivo* due to increased telomere breakage, both of which are at the basis of the BM failure syndrome [23–25].

Aside from the evidence discussed above that telomere length decreases in the hematopoietic system under steady state conditions, and more so under stress condition, there is now also evidence that HSC from young and adult animals differ in their functional and molecular characteristics.

A number of studies have addressed the number, phenotype and repopulation ability of HSC in young vs. old mice, and defined some of the genetic traits that affect aging of HSC. Already in 1996, it was found that, surprisingly, the number of cells with an HSC cell surface phenotype increases five fold in the BM of old C57Bl/6 mice [26]. These results were later confirmed in other studies [27,28]. However, the frequency of functionally defined HSC, i.e. cells that can compete with other cells for engraftment in an ablated animal, is relatively unchanged.

The decrease with age in the fraction of phenotypically defined HSC capable of *in vivo* repopulation may be explained by a number of factors. Some have suggested homing and subsequent engraftment of old HSC is decreased compared to young HSC [26]. Alternatively, Sudo et al., suggested that a higher fraction of phenotypically defined HSC in aged mice are actually not LTR-HSC but stem/progenitor cells that engraft mainly in the myeloid compartment that retained the classical HSC surface phenotype (cKit+Sca1+Lin-CD34-/low) [29]. Of note, stem/progenitor cells in older animals that contribute chiefly to the myeloid lineage could self-renew, as progeny isolated from the first recipients could reconstitute hematopoiesis in secondary animals, again favoring myeloid reconstitution, although lymphoid reconstitution was also seen. Yilmaz confirmed the finding by Sudo et al, demonstrating that old mice have a larger fraction of non reconstituting HSC than young mice, and that this population can be identified by differences in expression of SLAM receptors [29].

However, the increased number of LTR-HSC in aging C57Bl6 mice is specific for this strain of mice, as the number of LTR-HSC decreases with aging in other mouse strains [30]. This observation has been exploited to create congenic mice making forward genetics approaches possible to identify genetic loci involved in HSC regulation and/or aging phenotype [31–33]. Using the BXD recombinant inbred strain, Henckaerts et al. found 7 putative quantitative trait loci linked to HSC lifespan with 4 of them overlapping with the number, frequency or proliferative capacity of LSK cells [31]. In a similar study, Geiger et al. demonstrated that a locus on chromosome 2 regulates hematopoietic stem cell aging. The authors also linked the response to radiation to HSC aging, hypothesizing that defects in DNA repair mechanisms might underlie HSC aging [32]. Interestingly the locus on chr. 2 linked to HSC aging is in close vicinity to the one linked to HSC lifespan.

Others have compared the transcriptome of HSC from young and aged animals to understand mechanism(s) underlying aging of HSC. Rossi et al. used oligonucleotide microarrays containing 34,000 genes and found that 907 were significantly differentially expressed between HSC (defined as cKit+Sca1+Lin-Flk2-CD34-cells) from young and old mice. Sixteen genes more highly expressed in HSC from aged animals have been implicated in human leukemias. Such increased expression of key genes involved in hematopoietic malignancies would be consistent with the observation that humans treated extensively with chemotherapeutic agents develop MDS and leukemia, and the ultimate leukemic transformation seen in aging HSC in FA and DKC. Interestingly, lymphoid associated transcripts were enriched in HSC of young animals (30/43 differentially expressed), whereas myeloid associated transcripts were enriched in HSC of aged animals (29/38), consistent with the observations by Sudo et al. that repopulation from HSC from aged animals is skewed to the myeloid lineage [29]. In a similar study, Chambers et al., compared the gene expression profile of HSC defined as Side Population (SP) positive, cKit+Sca-1+Lin-cells from young and old mice. 3000 genes out of 14,000 were differentially expressed with aging. The functional categories of DNA repair, chromatin remodeling, and silencing genes were expressed less in HSC of aged animals. The authors also observed coordinated loss of transcriptional regulation in certain chromosomal regions with an overall increase in transcriptional activity including genes normally silenced by epigenetic mechanisms. These findings suggest that genetic and epigenetic alterations may be responsible for the observed differences in HSC with age [34].

The hypothesis that DNA damage is a major mechanism that alters the properties of aging HSC has been tested in mice with deficiencies in different DNA repair pathways and telomere

maintenance (Ku80$^{-/-}$, XPD^{TTD} and old generation mTR$^{-/-}$) revealing a decline in the overall functionality of LTR-HSC in these knock-out models [35]. Using mice with a deficiency in a DNA ligase, Nijnik et al. found an age-related decline in multipotent cells within the KLS population [36]. Furthermore, Rossi et al. showed increased number of H2AX foci (a marker of DNA damage response) in HSC of aging wild type mice compared to young mice. Intriguingly, they found that more committed HSC progeny in aged mice had less H2AX foci than the HSC themselves, suggesting either that the damaged progeny might be eliminated, that damaged HSC do not divide or that there exist better repair mechanisms in the progenitors than in HSC [35].

Other studies have found that functionality of aged HSC is affected in mouse models with deficiency in genes involved in response mechanisms to DNA damage and telomere shortening, suggesting that some of the same mechanisms that prevent the accumulation of damaged stem cells (anti-cancer mechanisms) might be involved in their decreased functionality or exhaustion with aging [37].

Aging of mesenchymal stem cells (MSC)

Mesenchymal stem cells, a.k.a. marrow stromal cells (MSC) were originally described as colony forming unit fibroblasts (CFU-F) by Friedenstein et al. as they can be purified from BM as plastic-adherent fibroblastic cells [38]. Later, it was found that MSC are a distinct population in the BM, co-existing with HSC, that has the potential to differentiate towards lineages of mesenchymal origin including bone, cartilage, fat, connective tissue, muscle and marrow stroma [3,39]. MSC have since been isolated from many tissues (e.g. adipose tissue, synovial fluid, periosteum, umbilical cord blood, several fetal tissues, …). MSC, owing to their trophic effects, their immunosuppressive effects, and their ability to generate osteocytes and chondrocytes are good candidates for the use in regenerative medicine.

Although MSC have been studied for three decades, the exact nature of the progenitor present *in vivo* is only now being characterized. MSC populations obtained by plastic adherence are initially very heterogeneous and contain probably only rare 'true' stem/progenitor cells. Different cell surface markers have been proposed to prospectively isolate MSC; however, little agreement has been reached on the use of a specific set of markers to define MSC *in vivo*. Recently, Bianco et al. demonstrated that self-renewing osteoprogenitors in human BM that regenerate bone and stroma, and organize a hematopoietic microenvironment *in vivo* are CD146^{High} (a melanoma associated cell adhesion molecule), possibly providing a positive selectable marker for MSC in fresh tissue [40].

As primary prospective isolation of MSC from tissue remains difficult, clinical use of MSC currently still depends on culture isolation followed by expansion. However, not only isolation methods, but also the culture conditions to select and expand MSC are highly variable, and the fate *in vivo* of cells following expansion is not fully studied. Thus, in contrast to HSC, due to the incomplete characterization of freshly isolated MSC and the variable nature of MSC following culture expansion, assessment of the effect of different variables, including aging, on the frequency, phenotype and expansion potential of MSC remains incomplete. We will here summarize what is known regarding the effect of aging (*in vivo* or *in vitro*) on MSC frequency, *in vitro* proliferation, and *in vitro*/*in vivo* differentiation potential.

Culture-expanded hMSC have spindle-shaped fibroblastic morphology and usually stop proliferating before or at 40 population doublings (PD's), at which time the cells become bigger and more flattened [41]. Hayflick et al. described already in 1961 that an increase in cell size is often associated with senescence *in vitro*. One of the mechanisms underlying cell senescence is loss of telomere length. A number of studies have measured telomere length of MSC, with varied results. Baxter et al. described, as would be expected, that telomere length in MSC from young donors is significantly longer than in older donors and the average loss *in vivo* could be estimated at 17 bp/year [42]. Upon *in vitro*-culture, they document progressive loss of telomere length with each cell division and found that MSC stop dividing when telomere length reaches about 10 kb. By contrast, Guillot et al. demonstrated that telomere length of MSC derived from postnatal donors is in the range of 7–8 kb, not different from any other postnatal cell; whereas telomeres of MSC derived from prenatal, primarily first trimester blood, have telomeres that range between 11 and 13 kb [43]. Bonan documented average telomere lengths of MSC derived from postnatal BM of 7–9 kb [44] whereas Mareschi et al. [45] found that telomeres in MSC from young donors were in the range of 10 kb. All studies document teleomere loss of up to 1.5–2 kb upon passage *in vitro*. The predictable and progressive loss of telomere length upon MSC passage *in vitro*, makes it possible to deduct from the mean telomere length of MSC at passage 2 the final number of PDs the MSC population will undergo [46]. From the study by Guillot et al. and Mareschi et al., among others, it is also obvious that MSC from younger donors have longer telomeres, allowing more protracted *in vitro* expansion.

As cell senescence is at least in part due to telomere length, and MSC, like all other adult (stem) cells, have low levels of telomerase activity [47], several groups have tested whether forced expression of hTERT would extend the lifespan of hMSC. hTERT-transduced hMSC have prolonged replicative capacity *in vitro*, as they undergo more than 80 and 260 PDs, respectively, whereas they retained adipo-, chondro- and osteogenic differentiation potential *in vitro* and osteogenic potential *in vivo* [48,49] Moreover, there was no evidence for tumor formation and the cells showed a normal karyotype. Thus, ectopic expression of hTERT in hMSC can maintain the proliferative and differentiation ability of human MSC. Whether this approach has clinical relevance to generate very large numbers of MSC is not clear.

Liu et al. isolated MSC from telomerase knock-out mice and noticed a complete failure of the cells to differentiate into adipocytes and chondrocytes, even at early passages [50]. Recently, a mouse model of accelerated aging was created by combining the telomerase knock-out mouse and a Werner knock-out mouse. The Werner syndrome is a well known premature aging syndrome characterized by telomere dysfunction. The double mutant mice showed age-related osteoporosis which was the result of declined osteoblast differentiation. Moreover, MSC had *in vitro* a shorter proliferative capacity and an impaired osteogenic potential, consistent with premature senescence [51].

However, shortening of telomeres is not the only factor to induce cellular senescence. Shibata et al. found that the expression of $p16^{INK4A}$ is strongly associated with senescence and that a prolonged cultivation of human MSC may inactivate this cell cycle regulator gene, leading to chromosomal aberrations [46]. Consistent with this notion is the finding that more abundant transcripts involved in cell cycle promotion, chromatin regulation and DNA repair are present in fetal than postnatal hMSC [52].

Aside from a decrease in overall expansion potential, several groups have also documented that MSC from older donors have a slower proliferation rate, already from the initial cell passage until the culture senesces [42,53–56]. Interestingly, this is associated with a different morphology of MSC from the initial passage, as MSC from older donors are larger and flatter compared with MSC from younger donors.

A third observation that follows from some studies is that the MSC frequency in BM samples from old donors (as measured by the CFU-F assay, a standard *in vitro* method to determine the MSC cell number in a sample) is significantly lower than that in young donors [42]. Others did not notice significant differences in CFU-F colony formation between donors from various ages; however in these reports the difference in age between the 2 donor groups is rather small and/or these studies did not include donors at very young ages (e.g. from 0–14 years) [57,58]. A more recent study, using a wider donor range, confirmed a decline in MSC numbers with age [59].

As has been done for HSC, a number of groups have assessed the influence of age (*in vitro* or *in vivo*) of MSC on their differentiation potential. One caveat, in contrast to HSC where functional assessment occurs following grafting *in vivo*, most studies have addressed differentiation of MSC *in vitro*, which may not, however, predict the potential of the cells *in vivo*. Different groups have studied the osteogenic differentiation capacity of MSC derived from young and old donors. The majority of the studies reported a decrease in the generation of alkaline phosphatase positive progeny from CFU-F according to the age of the donors [42,56,60]. However, Stenderup et al. found no difference in osteogenic differentiation capacity between MSC from young and old donors, either *in vitro*, or *in vivo*, following seeding hMSC on calcium phosphate scaffolds and implanting them subcutaneously in NOD/SCID mice [61]. Mendes et al. also found that MSC derived from donors of every age group were capable of forming bone *in vivo*, even though the frequency with what they could detect bone formation was significantly decreased with donor age [55]. A second cell type that can be generated by MSC is chondrocytes. Zheng et al. compared the chondrogenic capacity of MSC from 1-week, 12-weeks and 1-year old rats. Only MSC-derived chondrocytes from 1-week old rats produced abundant collagen-type II, consistent with the finding that transcripts for aggrecan and collagen II declined with increasing age. These results suggest an age-related loss of chondrogenic potential of MSC *in vitro* [62]. A similar decreased ability with age has been seen in rat MSC to repair an infarcted heart [63]. By contrast, the age of the donor may not affect the ability of MSC to repair tendons [64].

Some studies have evaluated the differentiation capacity towards the 3 main MSC lineages (adipo-, osteo- and chondrogenic) using MSC from human donors with various ages [65]. The frequency of bipotent clones (with osteo- and chondrogenic potential) decreased with age, whereas tripotent clones could be found in every age group and reached up to 23 population doublings, but only maintained their original differentiation potential up to 19 doublings. Interestingly, the *in vitro*-cultured MSC clones lost first their adipogenic potential. This is in contrast with the *in vivo* hypothesis on aging MSC, where there is an enhanced adipogenesis and a decreased osteogenesis [66].

Hence, as for HSC, the frequency as well as proliferative and differentiation potential of MSC appears to be affected by age, even though analysis of MSC is hampered by the fact that they cannot yet be prospectively isolated, and because their function is mostly evaluated using *in vitro* studies.

BM derived stem cells with extended differentiation capacity

Since 2002, several groups have isolated cells from BM or UCB that, like MSC, can be cultured *in vitro* adherent to plastic, named MAPC [67], hBMSC [68], USSC [69], FSSC [70], AFS [71], MIAMI cells [72], hFLMPC [73], and MASC [74]. In contrast to MSC and HSC described above, MAPC, hBMSC, pre-MSC and other cells described here have the ability to generate cells of multiple germinal layers, including mesenchymal cells as well as endothelium, hepatocyte like cells and neuroectoderm-like cells. In addition, a number of these cell populations, including MAPC, hBMSC, MIAMI cells, AFS and MASC, may be capable to proliferate without telomere shortening. Many of them are reported to express the ES cell specific transcription factor Oct4 (and Nanog); they lack HSC cell surface marker expression and express a variable complement of cell surface antigens found on MSC. Despite expression of Oct4 these presumed more potent adult stem cells lack other typical ESC pluripotency characteristics such as formation of EBs and teratomas. It remains unclear whether MAPC, hBMSC, USSC, FSSC, AFS, MIAMI cells, and MASC exist *in vivo*, or are a cell phenotype induced by culture, leading to possible reactivation of genes that endow them with greater differentiation and proliferation potential. Lengner et al. elegantly demonstrated that deletion of Oct4 in stem cell compartments that do not normally express this transcription factor *in vivo* such as HSC, MSC and other adult tissues did not result in reduction of their functionality [75]. This might signify that even if cells with extended differentiation ability exist *in vivo*, they may play little or no role in spontaneous healing processes.

Lengner et al. also demonstrated, using a mouse wherein IRES-GFP was knocked in behind the 5th exon of Oct4, that cells expressing GFP, representative of Oct4 expression at the level of ESC, cannot be identified in postnatal somatic tissues, suggesting that Oct4 expressing cells found in culture may be a culture induced phenomenon [75]. However, Anjos-Afonso et al., isolated CD45-CD31-SSEA-1+ pre-MSC from BM mononuclear cells of mice that express low levels of Oct4 and Nanog, suggesting that cells expressing Oct4 may exist, although levels are low [76]. Interestingly, Oct4 expression was upregulated >100 fold *in vitro* following expansion in MAPC culture medium [67], which would be consistent with the notion of culture mediated de-differentiation of cells, perhaps akin to what has been described for iPS cells. Using the pre-MSC surface phenotype to identify them

in fresh BM of mice at different ages, this group found a drastic reduction in the number of cells with this phenotype from 0.03% in 2 week old mice to <0.01% in 4 week old mice, and a further reduction <0.001% in 1-year old mice. Whether the differentiation capacity of pre-MSC is affected by age was not addressed.

Concluding remark

Loss of self-renewal and acquisition of defects in differentiation of stem cells is at least partially responsible for the typical phenotype of the aging process. The molecular mechanisms that contribute to aging of stem cells are also those involved in the elimination by apoptosis or senescence of cells with genetic damage, which could pose a risk to the organismal integrity.

Both HSC and MSC are currently being used clinically. Hence, understanding aging of these stem cell compartments is not only important from the perspective of preventing or ameliorating their aging associated dysfunctionality *in vivo*, but also should be taken into account when selecting donors for stem cells to be used in cellular therapies. HSC are the best characterized stem cells and we have a reasonable understanding of the effect of age on the proliferative and functional abilities of HSC. For instance, this has lead to the increasing use of HSC from neonates, specifically umbilical cord blood, in the clinic. Studies on MSC suggest that as for HSC, MSC undergo a decline in their differentiation and expansion capacity with organismal aging; however, the extent of this decline is not clear due to lack of consensus on MSC phenotypic characteristics, growth conditions and the reliance on *in vitro* studies preclude extensive comparisons. Improved understanding of these age induced effects on MSC will no doubt also impact their clinical use in the future.

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