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Aging of marrow stromal (skeletal) stem cells and their contribution to age-related bone loss

Ilaria Bellantuono [a,*], Abdullah Aldahmash [b], Moustapha Kassem [b,c,*]

[a] Academic Unit of Bone Biology, University of Sheffield Medical School, Beech Hill Rd, Sheffield S10 2RX, UK
[b] Stem Cell Unit, Department of Anatomy, King Saud University, Riyadh, Saudi Arabia
[c] University Department of Endocrinology, University Hospital of Odense & Medical Biotechnology Center, Winslowsparken 25, DK-5000 Odense, Denmark

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ABSTRACT

Marrow stromal cells (MSC) are thought to be stem cells with osteogenic potential and therefore responsible for the repair and maintenance of the skeleton. Age related bone loss is one of the most prevalent diseases in the elder population. It is controversial whether MSC undergo a process of aging in vivo, leading to decreased ability to form and maintain bone homeostasis with age. In this review we summarize evidence of MSC involvement in age related bone loss and suggest new emerging targets for intervention.

1. Introduction

Human aging is associated with bone loss leading to bone fragility and increased risk for fractures, a disease known as osteoporosis. Osteoporosis is one of the most prevalent and serious diseases affecting the elderly population and constitutes a major public health problem. The cellular and molecular causes of age-related bone loss are currently intensive topics of investigation with the aim of identifying new approaches to prevent and treat osteoporotic bone loss. The aim of this review is to summarize the recent literature that relates senescence of stromal (also known as mesenchymal stem cells and abbreviated MSC) to aged-related decrease in bone formation.

2. The concept of bone remodelling in relation to MSC

Bone as a tissue, is composed of bone matrix and bone cells. Two main types of mature bone cells have been identified: osteoblasts (bone forming cells) and osteoclasts (bone resorbing cells) [1]. The main function of those cells is to mediate a bone replacement mechanism called "bone remodelling" aiming at maintenance of the integrity of the skeleton by removing old bone of high mineral density and high prevalence of fatigue micro-fractures and replacing it with young bone of low mineral density and better mechanical properties [1,2]. Bone remodelling comprises a specific sequence of cellular events. It starts with recruitment of osteoclast precursors, their fusion to form mature osteoclasts followed by their migration to bone surfaces. The osteoclasts remove an amount of bone and create a resorption lacuna. Bone resorption is followed temporally in the same anatomical location by bone formation. Key to the initiation of the process of bone formation is the recruitment of osteoblasts from stem cells and precursor cells in the bone marrow and their migration to the bottom of the resorption lacuna. Osteoblasts deposit matrix that mineralizes and forms lamellar bone. Usually the amount of bone removed by the osteoclasts is equal to the amount of bone formed by the osteoblasts and a stable bone mass is maintained. However, this is not the case during aging. Increased bone resorption and/or decreased bone formation can lead to bone loss. Based on a number of histomorphometric studies performed on iliac crest biopsies, a decrease in bone formation seems to be the principal pathophysiological mechanism responsible for age-related decreased bone mass [2].

2.1. Mesenchymal stem cells and their contribution to bone formation

Bone formation is dependent on the number and the activity of osteoblasts recruited at bone formation sites during bone remodelling. It is assumed that osteoblasts are differentiated from osteoprogenitor and stem cells present in the bone marrow. Based on the pioneer work of Friedenstein and co-workers [3], it has been recognised that the non-hematopoietic compartment of bone marrow (known as bone

* Corresponding authors. I. Bellantuono is to be contacted at Room DU19, Academic Unit of Bone Biology, Faculty of Medicine, Dentistry and Health, University of Sheffield, Beech Hill Rd, Sheffield S10 2RX, UK. Tel.: +44 114 271179; fax: +44 114 2711711. M. Kassem, Odense & Medical Biotechnology Center, Winslowsparken 25, DK-5000 Odense, Denmark.
E-mail addresses: i.bellantuono@shef.ac.uk (I. Bellantuono), mkasem@health.sdu.dk (M. Kassem).

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marrow stroma) contains a group of fibroblast-like stem cells with osteogenic differentiation potential. In vivo transplantation of MSC in syngeneic or immunodeficient mice in ectopic sites resulted in the formation of a mixture of tissues including bone, cartilage and hematopoietic-supporting stroma [3,4]. However, the identity of these cells, and whether they hold true properties of stem cells is still matter of debate (see below).

3. Biological characteristics of MSC

3.1. Are MSC stem cells?

Stem cells are defined by their ability to reconstitute in vivo the tissue of origin giving rise to all the differentiated mature cell types present in that tissue and contributing to its long term maintenance and repair. Stem cells differ from progenitor cells (also known as transient amplifying cells). Progenitor cells exhibit extended proliferative capacity and multipotent differentiation ability in vitro, but have very little ability to contribute to long term tissue regeneration in vivo. In contrast, to demonstrate "stemness" properties, cells have to show the ability to regenerate the tissue of origin following transplantation and possibly in serial transplantation studies. There is very little evidence for long term skeletal regeneration capacity of MSC in vivo. Transplantation of MSC by intravenous (iv) infusion has shown disappointing results with low or no engraftment of the cells to bone and bone marrow [5–8]. Inefficient migration has been thought as the cause of lack of engraftment [6,9]. However, intra-femoral injection of a subset of murine marrow stromal cells showed limited engraftment at the site of injection 4–6 weeks post-transplantation with few cells expressing osteoblastic markers [10], suggesting that the quality of the cells injected may be the main cause of low engraftment.

More encouraging are the studies testing the preservation of long term self-renewal by implantation of MSC in heterotopic sites [4,11]. In a recent study by Sacchetti et al. [12], implantation of a subpopulation of MSC, which are $CD45^{low}/CD146^{bright}$, regenerated bone and stroma-supporting hematopoiesis in ectopic bone formation assay. Most importantly highly clonogenic CFU-F were re-isolated from the implants, suggesting that some of the implanted MSC underwent self-renewal in vivo. In the study by Quarto et al. MSC isolated by plastic adherence and transduced with an erythropoietin cDNA, were transplanted (along with hydroxyapatite particles) under the skin of nude mice. The cells were able to form bone and bone marrow stroma and the animals maintained increased hematocrit levels for 12–14 weeks [13]. More interestingly MSC were re-isolated from the implants and used in secondary transplantation showing sustained increased hematocrit levels similar to those obtained in primary transplantation. These studies provide a proof of principle that a stem cell with osteogenic potential exists among MSC.

3.2. Identification of MSC

The paucity of studies demonstrating stem cell properties of MSC in vivo highlights the difficulties in the isolation and identification of the true MSC with self-renewing and multipotential differentiation abilities. The absence of suitable markers to establish a hierarchical relationship between stem and progenitor cells has left scientists with a variety of protocols for the isolation of MSC, which vary from laboratory to laboratory and rely mostly on physical–chemical conditions of the in vitro culture. The most popular method for isolating MSC from bone marrow is through plastic adherence [14,15]. The resulting MSC cultures are heterogeneous and contain a mix of progenitors at different stages of commitment [15,16].

In vitro, MSC are identified retrospectively by their clonogenic growth capacity (known as colony forming unit-fibroblast CFU-F) and their ability to differentiate into a broad spectrum of fully differentiated connective tissues, including cartilage, bone, adipose tissue and myelosupportive stroma in vitro [17]. However, remarkable differences have been observed among the different CFU-Fs in terms of cell morphology, rate of replication, expression of markers for osteoblastic, chondroblastic, adipogenic phenotypes and the number of differentiated progeny they can give rise to, with some CFU-F capable of multipotent differentiation, others only capable of forming bone and yet others capable of giving origin to myelo-supportive stroma [15,16,18–21].

Unfortunately, there are no commonly accepted surface antigens, which can be used to prospectively isolate MSC from bone marrow. To-date the markers used are not specific to stemness characteristics and are common to several mature cell types. MSC are recognised as negative for CD45 (non-hematopoietic) CD34 (non-endothelial) and positive for CD90, CD105 (SH2), CD73 (SH3), CD63, Stro-1 [22,23]. There have been several attempts to identify novel markers for prospective isolation of MSC with stem cell properties (Table 1). Stro1 is among the first and one of the most investigated marker. Stro-1 antibody identifies a glycoprotein component on all the clonogenic MSC [23]. However expression of Stro1 is not unique to MSC as it is also expressed in erythroid precursors. Transplantation of Stro1+ cells in vivo did not show improved engraftment potential [7]. Moreover, there has been no evidence that Stro1+ cells have the ability of in vivo self-renewal. It remains unclear whether Stro1 identifies a population of cells, which is more primitive compared to cells isolated by plastic adherence, and whether the more recently isolated $CD45^{low}/CD146^{bright}$ subpopulation of MSC [12] (see above) performs better in serial transplantation. A turning point in the establishment of a hierarchical relationship within the BMSC may be represented by a recent study by Nishikawa's group [24]. The authors examined the developmental origin of MSC, using murine ES cell as model. A very early progenitor cell, the $Sox1^{+}$ neuroepithelial cell was identified as the primary cell of origin of MSC. $Sox1^{+}$cells

Table 1
Markers used to perspectively isolate MSC from bone marrow

Marker	Cross reaction	% cells generating CFU-F	In vivo bone formation	Re-isolation following transplant	Telomerase expression
Stro-1 [23,43,74]	Erythroblasts, B cells subset	9	Bone and hematopoietic microenvironment	N/D	Yes
Stro-1/VCAM [43]	B cells	50	Bone formation in 35/64 clones Hematopoietic microenvironment in 11/35 clones	N/D	Yes
CD105 [75]	Endothelial cells, pre B leukemic cells	0.006	N/D	N/D	N/D
CD271 (LNGFR) [76,77]	Neural cells	0.16–1.9	N/D	N/D	N/D
$CD45^{low}$ D7 Fib/CD271 [78]	D7Fib-skin fibroblasts	15	N/D	N/D	N/D
SSEA4 [79]	Embryonic (carcinoma) stem cells, germ cells	N/D	Bone formation	N/D	N/D
GD2 [80]	Neural cells	N/D	N/D	N/D	N/D
CD45-CD146+ [81]	CD146-T cells, melanoma, endothelial cells	2.6	Bone formation and hematopoietic microenvironment in 2/4 clones	CD146+ cells and CFU-F	N/D

N/D, not done.

subsequently became cells with properties of MSC progenitors through the sequential acquisition of PDGFRα and PDGFRβ expression. It is unknown if any of the cells emerging from the Sox1$^+$ PDGFRα and PDGFRβ populations were CD45^{low}/CD146^{high} or whether any of these intermediate cell populations are present in the post-natal human bone marrow.

4. Age-related changes in MSC

As mentioned above, stem cells are required for the continuous supply of mature, functionally competent cells for normal tissue turnover and regeneration. An age-related decrease in the number or functional abilities of stem cells is thought to lead to tissue deterioration. In the following we will review evidence that MSC exhibit changes in their biological characteristics during aging that are relevant for their bone forming abilities.

4.1. Age-related changes in size of the MSC pool

Considering the lack of appropriate markers and assays to identify MSC it is not surprising that no agreement has been reached as to whether MSC decrease in numbers with age and whether this has a role in the decrease bone mass observed during aging. Several investigators have utilized the in vitro clonogenic ability of CFU-F to determine the number of MSC progenitors. Others have measured the clonogenic ability of their osteoblastic-committed populations, the CFU-O, which is alkaline phophatase positive and is obtained following exposure of bone marrow cells to osteogenic supplements. In rats a decrease in MSC number is reported but this is not consistent in murine MSC (Table 2). The reason for this discrepancy may be related to the experimental method utilized. In the murine studies, the number of CFU-F has been corrected for the number of cells present in the femur. In contrast in the rat and human studies, CFU-F or CFU-O has been calculated as frequency of bone marrow mononuclear cells. Moreover, age-related effects on MSC may be strain dependent. This is well known from studies performed on hematopoietic stem cells (HSC), where the repopulation ability of HSC is compromised with age in Balb/c but not in C57Bl/6 [25]. Similarly, femoral bone density decreases with age in Balb/c but not in C57Bl/6 [26] suggesting that Balb/c mice can be more sensitive to age related changes. Unfortunately most of the studies to assess MSC numbers have been carried out in C57Bl/6 strain. Interestingly, the only study partly carried out using Balb/c showed a decrease in the number of CFU-F and CFU-O [82].

Discrepancies exist also in the human studies that examined the effect of age on the number of CFU-F or CFU-O [27–34]. It is plausible that these inconsistencies in the results are due to differences in the methods and source of bone marrow samples utilized. Some studies used bone marrow obtained during surgical procedures for treatment of osteoarthritis or other operations, which may not represent the bone marrow of a healthy aging population. Also, bone marrow was obtained from different sites (spine, proximal knee, iliac crest) and with different techniques. Some authors used bone marrow aspirates and other used "flushing" technique to remove bone marrow cells from the bone fragments obtained during orthopaedic procedures. Methods of collection of bone marrow aspirates can influence widely the number of MSC present depending on the extent of blood contamination. Moreover, the site of harvest may determine the type of MSC obtained. Sacchetti et al. [12] showed that the more primitive CD45^{low}/CD146^{high} MSC is contained in the bone marrow obtained from the iliac crest and not from cells from the periosteum or trabecular bone. Another important factor, which can influence the outcome of the studies, is the way a CFU-F or a CFU-O is defined. Some investigators defined a CFU as a group of cells with more than 16 cells whilst other more then 50 cells. It is of interest that only the studies where colonies with more than 50 cells were counted showed a decrease with age (Table 2) and it raises the question whether these two colony types represent different cell populations with different biology.

In an attempt to overcome some of these experimental limitations, Stenderup et al. [33] examined the number of CFU-F and CFU-O present in Stro-1$^+$cells isolated from bone marrow mononuclear cells in 38 normal healthy volunteers (23 young and 15 old individuals) and 13 patients with osteoporosis. No age-related effects on the total number of CFU-O, CFU-F or CFU colony size was observed [33]. In contrast, the number of CD45^{low}/D7fib$^+$/LNGF$^+$ cells was reported to decrease with age and was positively correlated to the number of CFU-F [34]. However, the difference between the two studies may be

Table 2
Summary of studies assessing age-related changes in mesenchymal stem cell numbers, Y = young donor, O = older donors, F = Female, M = Male, N = Number, mo = months, y = years, OA = Osteoarthritis

Specie	Age	Assay	Outcome	Reference
Rats	6/12 mo (Y), 24 24 mo (O)	CFU-O	Decrease	[29]
Rats	4 mo (Y) 21 mo (O)	CFU-F CFU-O	30% decrease CFU No difference in CFU	[28]
Mice	4/6 mo (Y), 24 mo (O)	CFU-F CFU-O	Decrease in CFU-F and CFU-O	[82]
Mice	3 to 15 mo	CFU-F (>50 cells)	Increase	[83]
Mice	3 to 15 mo	CFU-F (>50 cells)	No increase if corrected for increase in cellularity	[84]
Rats	5–6 weeks (Y), 18 mo (O)	Bone nodule in culture Proliferation rate	Decrease bone nodule No change in proliferative rates	[85]
Human	4 to 88 y (49 females)	CFU-O (>50 cells)	Decrease	[86]
Human	13–88 y (57 donors)	CFU-O (>8 cells)	No difference	[87]
Human	Only females (26 donors)	CFU-O (>8cells)	Decrease	[87]
Human	3–36 y (5 F and 14 M) 41–70 y (11 F and 11 M) BM from vertebrae	CFU-O (>50 cells)	Decrease up to 40 y	[88]
Human	17–87 y (28 donors, BM sternum)	CFU-F (>128 cells)	Decrease up to 30 y	[30]
Human	17–87 y (28 donors, BM sternum)	CFU-F (<32 cells)	No change	[30]
Human	14–48 y (16 donors) 28–87 y (57 patients with OA) 69–97 (26 patients with osteoporosis)	CFU-F CFU-O (Size in mm^2)	No difference	[31]
Human	1–18 y (Y) and 59–76 y (O)	CFU-F (>50 cells)	Decrease	[32]
Human	22–44 y (10 F and 13 M) 66–74 (15 F) 58–83 y (12 F with osteoporosis)	Stro1+/CFU-F (>16 cells) Stro1+ CFU-O (16 cells)	No difference with age or osteoporosis	[33]
Human	5–55 y (33 donors)	CFU-F (>50 cells) CD45lowD7fib+CD271+	Decrease	[34]

related to the age characteristics of the donors employed. The change in the number of MSC with age was observed in the studies when a higher number of young donors were included, suggesting that most of the observed decline in the number of CFU-F occurs in early adulthood when changes in the skeletal dynamics from a modelling mode characteristic of skeletal growth and consolidation to a remodelling dynamics characteristic of the adult skeleton occurs. This is true for the study by Stolzing et al. [34] where the major difference in CFU-F numbers occur between the adolescent group (age below 20) and adult group (age above 20). This may also explain why experiments employing rats showed a decline in the CFU-F number as they continue to grow throughout their lifespan. Changes later in life may be very small if at all, possibly present only in certain pathological conditions. Similar observations have been reported in HSC where the highest proliferative demands are present in the first year of life and changes after that are very small and difficult to detect unless in condition of high proliferative stress such as following transplantation (reviewed in [35]).

4.2. Age-related changes in proliferative capacity of MSC

Due to the very property of self-renewal, the proliferation ability of stem cells is expected to be extensive or even indefinite. The effect of donor age on the maximal proliferative potential of plastic adherent bone marrow-derived MSC has been examined by several investigators. An age-related decline in the maximal life span from 30–40 population doublings in younger donors to 20 PD in older donors was observed [32,36] suggesting that MSC lose proliferative capacity in vivo as well as in vitro. This is mirrored by telomere shortening and increased number of MSC showing signs associated with senescence such as a change in morphology (from spindle shape to large flat cells) [32,34,36], expression of cell senescence markers e.g. β galactosidase, tumor suppressor TP53 and cell cycle regulator protein p21 [34,36] and an increase in the levels of oxidized and glycated protein and lipofuscin, metabolites present in senescent cells [34].

Several studies have demonstrated a significant decrease in the mean telomere restriction fragment (mTRF) in MSC obtained from younger donors between the primary passage and the end of the culture, which significantly correlated with the number of population doublings [18,32,37,38]. Most importantly, when MSC were cultured for an equal number of population doublings, the telomere length in younger donors was significantly longer compared to older donors [32,36]. If one assumes that all MSC undergo telomere shortening at similar rates in vitro, these data imply that the difference in telomere length seen between younger and older donors is due to loss of mTRF occurring in vivo and can be estimated to be about 17 bp/year [32]. Measurement of telomere length in uncultured MSC is now required to exclude that culturing of MSC of young and old donors does not introduce a bias and that the difference in telomere length seen is truly a reflection of a loss occurring in vivo. However the fact that the telomere length of MSC obtained from fetal blood, liver and bone marrow was significantly higher ($p<0.01$) compared to adult MSC [39], shows the loss of telomere length with developmental age and is well in agreement with these data.

Theoretically, long-term growth of MSC requires maintenance of telomere length that is usually accomplished by the presence of telomerase activity an enzyme which adds telomeric repeats at the end of chromosomes. In HSC expression of telomerase has been shown to delay the process of aging although it did not prevent it [40]. It is controversial whether MSC express telomerase to sufficient levels to act in a similar way. Most of the studies agree that telomerase is not expressed by MSC in culture [41,42]. However, only one study suggested that telomerase may be expressed in a sub-population of MSC expressing STRO-1^{bright} VCAM+ marker before their exposure to in vitro culture conditions [43]. It is possible that, similarly to HSC, a very primitive subpopulation of MSC expresses telomerase in vivo. However, upon isolation in culture, MSC lose part of their stemness and telomerase is switched off. In support of this a very recent study demonstrated that culture expanded human MSC underwent commitment towards osteoblast lineage and expressed high levels of osteogenic regulatory gene Cbfa1, and alkaline phosphatase. Low levels hTERT transcripts and telomerase activity were detected at the start but repressed by increased Cbfa1 expression [44].

4.3. Age-related changes in differentiation potential of MSC

Several studies have examined the effects of donor age on the differentiation potential of MSC. One caveat to these studies is that, in contrast to HSC, where assessment occurs following in vivo transplantation, in vitro culture and expansion of MSC is a prerequisite for performing differentiation studies and in several of these studies the degree of in vitro culture prior to differentiation studies has not been controlled. Their differentiation potential to the osteogenic lineage has been the most investigated in relation to aging. Stenderup et al. [33] and Justesen et al. [45], cultured MSC from younger, older and osteoporotic patients and found maintained osteoblast differentiation potential at early passage in culture (low population doublings). Similarly, Muraglia et al. tested the osteogenic, chondrogenic and adipogenic differentiation capacity of MSC in donors of various ages and found that the number of tripotent clones did not change with age in culture at early passage. However with time in culture the tripotent clones lost their adipogenic potential [21].

Some studies have examined the effect of donor age on bone forming capacity using in vivo bone formation assays. In this assay MSC mixed with hydroxyapatite/tricalcium phosphate (HA/TCP) as a carrier were implanted subcutaneously in syngeneic animals. Both cells from younger and older donors were tested in both younger and older recipient animals. MSC derived from aged rats exhibited decreased bone formation capacity compared with MSC from younger donors [46]. Stenderup et al. [11] employed similar methodology to test the difference between MSC obtained at early passage (few population doublings) from younger and older human donors. After implantation in immune-deficient mice, no difference was found in the amount of bone formed between the two age groups. On contrast, Mendes et al. [47] demonstrated that when human MSC from 53 donors of various age were seeded on calcium phosphate scaffolds and implanted under the skin of nude mice, the ability of cultures for forming bone declined with age with 67% of the culture able to form bone at 41–50 years of age, 50% at 51–70 years of age and less that 46% beyond 70 years of age. In conclusion, all these studies suggest that the age-related changes of MSC capacity for osteoblast differentiation and bone formation using early passage (low population doublings) of MSC are subtle and not consistent and may need sensitive assays for its detection. However, the effect of donor age becomes clearer upon in vitro expansion of the cells in culture due to an accelerated senescence phenotype. These findings are of relevance to regenerative medicine protocols where cells from elderly donor need to be used for therapy.

As mentioned above MSC can differentiate into osteoblasts and other lineages including adipocytes. In vivo, several investigators have demonstrated that with age there is a decrease in trabecular bone volume (TBV), which is associated with increased bone marrow adipocytic volume [48,49] and these changes are more pronounced in osteoporotic patients. Furthermore, in a mouse model of accelerated senescence (SAM-6 mouse) decreased bone formation was associated with enhanced adipogenesis in vivo and in vitro [50]. All these findings led to the hypothesis that, MSC acquired a bias in the differentiation ability in favour of the adipocyte lineage instead of the osteoblastic lineage. Although initial studies on the in vitro differentiation potential of MSC have reported that osteoblast differentiation was inversely correlated to adipocyte cell differentiation [51,52], these were superseded by numerous other studies where either no difference was seen or MSC from older donors exhibited a decreased

differentiation ability to the adipogenic lineage [32,34,45] when compared to younger donors. Thus, these studies suggest that adipogenic differentiation from MSC can decline with age in vitro but in vivo adipogenesis may be regulated independently and may not be necessarily related to impaired differentiation of MSC.

4.4. Age-related changes in bone microenvironment

Growth factors and cytokines are secreted in the bone microenvironment, sequestrated in bone matrix and exert their main effects locally as a controller of osteoblast activity during bone remodelling. Several investigators measured the effect of donor age on the amount of growth factors present in the bone matrix directly or indirectly through bone inductive effects of demineralised bone matrix powder. IGF-I and IGF-II are important regulators of osteoblastic cell proliferation and differentiation. Seck et al. [53] examined the relationship between donor age, cortical bone content of IGF-I and IGF-II as well as cortical bone remodelling in a large number of donors at different ages. Bone samples were obtained from the proximal femur neck. The authors found an age-related decrease in bone matrix concentration of IGF-I in both men and women and of IGF-II in men only. However, there was no significant correlation between bone content of IGF-I, IGF-II and any parameter of bone remodelling. From the same group, production of transforming growth factor (TGF)-β did not change with donor age [54].

Demineralised bone matrix (DBM) powder was implanted subcutaneously in animals of different ages and the amount of bone formed was quantitated using histomorphometric or biochemical techniques. Irving et al. [55] found that bone induction capacity of bone matrix is impaired in older animals. Bone powder obtained from tibiae and femurs of 6-month-old rats was implanted subcutaneously in young (6 weeks), adult (6 months), and old (2 years) rat recipients. Bone was formed after 14 days in young rats, after 15 days in adult rats and after 25 days in old rats. Similarly, the bone induction of powder obtained from bones of 4-month-old rats was dependent on recipient age with gradual decline in rats aged 1 month old to 16 months old [56]. Also, decreased in rates of bone formation and amount of bone obtained after subcutaneous implantation of DBM powder were observed in old rats (4 months old) compared with young rats (4 weeks old) [57]. The decreased bone formation found in rats was confirmed in rabbits where 3, 6, 18, and 28 months old rabbit recipients received intra-muscular implantation of DBM powder from 6 month-old male rabbit donors [58]. The amount of new bone formed was negatively correlated with age of the recipient. Similar experiments were performed using human DBM powder from younger (8 donors, 18–46 years) and older donors (9 donor, 62–90 years) that were implanted subcutaneously in mice [59]. The amount of bone formed by older donor DBM powder was decreased compared with younger donors but the difference was not statistically significant. These studies suggest that age-related changes in the bone microenvironment may play a role in the decreased bone formation occurring with age. However, it is unclear what cells are responsible for the change in growth factors secretion. It is possible that stem cells as such change their cytokine profile secretion to modify the environment to suite their survival, proliferation and differentiation needs. In an expression profiling study Cairney et al. (in this issue) have shown increased secretion of chemokines such as IL8 in HSC with age. MSC are known to secrete cytokines such as IL6, or IL11 which have been shown to change with age [60]. It is therefore possible that MSC exert an influence on the changes in the microenvironment and in turn on the decreased bone formation. Recently, we have employed serum as a surrogate for the microenvironment "seen" by MSC. We compared the effect of sera obtained from young and old donors on MSC proliferation and differentiation [61]. We found that sera of elderly donors were inhibitory to osteoblast and not adipocyte differentiation of MSC. The putative factor(s) responsible for these effects remain to be determined.

5. MSC aging and intervention to abolish age-related bone loss

Replacing or "rejuvenating" resident MSC populations by either MSC transplantation or by activation of resident MSC by bioactive molecules are potentially useful approaches to enhance the functional capacity of MSC and represent a novel approach to increase in vivo bone formation. During the recent years specific signaling pathways have been identified that provide possible target for intervention. Insight into these pathways has been obtained from studying human diseases of progeria (or accelerated senescence) and through combination of genetic studies and studying gene modified animal models.

5.1. Telomerase and rejuvenation of MSC

As we described above, cultured MSC lack telomerase activity and exhibit telomere shortening in culture. Also, telomere shortening was associated with expression of a senescence phenotype in MSC cultures [34,36]. We have tested the possibility of "rejuvenation" of MSC by forced expression of hTERT [42] and found that these telomerized MSC exhibit enhanced bone-forming activity both in vitro and in vivo besides promoting extended proliferation and differentiation ability of MSC [42,61]. We have also reported recently [62] that telomerase activity can be restored transiently to MSC by treatment with TSA (trichostatin A) that changes the epigenetic status of hTERT protomer. Unfortunately, long-term telomerization of the MSC leads to genetic instability and tumor formation [62]. Thus, alternative, short-term or conditional activation of telomerase may be a better approach for clinical intervention that remains to be examined.

5.2. Identification of Notch signaling in MSC aging

The identification of new molecular players involved in MSC aging came from the studies on progeroid syndromes. Progeroid syndromes (or accelerated aging syndromes) are caused by a single gene defects which results in accelerating aging phenotypes. The advantage is that aging features seen in such syndromes may result from the acceleration of mechanisms postulated to play causal role in aging and thus can give information on the contribution of a given mechanism in normal aging. In progeroid syndromes where stem cells have been studied a clear association among accelerated telomere shortening, decrease number of stem cells, organ failure and lifespan has been shown [63,64,66,67]. Werner syndrome (WS), dyskeratosis congenital (DC) and Hutchinson-Gilford Progeria syndrome (HGPS), are good example of this in the case of MSC [65,66]. In HGPS, a rare, fatal genetic disorder that is characterized by segmental accelerated aging with osteoporosis and accelerated telomere shortening, a link between BMSC and osteoporosis has been made. The major causal mutation associated with HGPS triggers abnormal messenger RNA splicing of the laminin A gene. Knock down of laminin A in BMSC has been shown to impair osteoblastogenesis and accelerates osteoclastogenesis [67]. Gene expression profiling of MSC overexpressing the abnormal form of laminin A showed alterations in the activation of the Notch signaling pathway [68]. Moreover, the abnormal form of laminin A has been seen to increase in cells with physiological aging [69], suggesting that, at least in certain circumstances, with age MSC may be affected in a similar way to MSC from patients affected by HGPS. Indeed Notch signaling has been shown to be important for the maintenance of the MSC pool in physiological conditions [70]. The disruption of Notch signalling in the limb skeletogenic mesenchyme markedly increased trabecular bone mass in adolescent mice whilst in aged mice caused osteopenia [70].

The identification of Notch signalling as a pathway involved in self-renewal of MSC opens up opportunities for drug discovery and strategies to identify compounds which can be used to manipulate stem cells for therapeutic or biotechnology purposes. Whilst considerably more information is needed, progress is well underway with proof-of-concept approaches. The ability of intervening and increasing even modestly the stem cell pool may have great therapeutic impact. Small molecule manipulation of both ES cells and adult tissue stem/progenitor cells has shown that such an approach is feasible [71–73]. A better understanding of the molecular basis of MSC aging may uncover novel molecule capable of extending tissue survival and repair.

6. Concluding remarks

The inadequate and very variable technology available at present for studying MSC and the diversity of mechanisms leading to aging pose a major challenge to studying the contribution of MSC to the aging phenotype and have led to the very variable results obtained in different laboratories. Studying larger samples of the healthy aging population is needed for an accurate analysis. Also, identification of better markers for the authentic MSC and a more controlled procedure for bone marrow harvest and culture are important pre-requisites for obtaining accurate data. Irrespective of these difficulties, the idea of "rejuvenation" of stem cells either through transplantation or activation of endogenous stem cells is highly relevant and may provide a novel strategy for abolishing some of the age-related diseases. In this context, studying the molecular mechanism underlying several of the progeria (accelerated-senescence) syndromes can enhance our understanding of the mechanisms of aging and MSC aging and thus provide novel targets for intervention.

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