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Age-related Changes in Bone Marrow Mesenchymal Stromal Cells: A Potential Impact on Osteoporosis and Osteoarthritis Development

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Abstract

Aging at the cellular level is a complex process resulting from accumulation of various damages leading to functional impairment and a reduced quality of life at the level of the organism. With a rise in the elderly population, the worldwide incidence of osteoporosis (OP) and osteoarthritis (OA) has increased in the past few decades. A decline in the number and "fitness" of mesenchymal stromal cells (MSCs) in the bone marrow (BM) niche has been suggested as one of the factors contributing to bone abnormalities in OP and OA. It is well recognized that MSCs *in vitro* acquire culture-induced aging features such as gradual telomere shortening, increased numbers of senescent cells, and reduced resistance to oxidative stress as a result of serial population doublings. In contrast, there is only limited evidence that human BM-MSCs "age" similarly *in vivo*. This review compares the various aspects of *in vitro* and *in vivo* MSC aging and suggests how our current knowledge on rejuvenating cultured MSCs could be applied to develop future strategies to target altered bone formation processes in OP and OA.

Keywords

aging, bone marrow (BM), mesenchymal stromal cells (MSCs), *in vitro*, *in vivo*

Introduction

Aging is a gradual process marked by the deterioration of functionality in living organisms with the passage of time. It is a complex phenomenon known to be affected by a variety of factors like diet, lifestyle, environment, heredity, and disease. It slows down the biological mechanisms that aid tissue maintenance, immunity, and health. Disorders like osteoarthritis (OA), osteoporosis (OP), Alzheimer's, and Parkinson's diseases have been associated with aging, making the life of patients more challenging as compared to a healthy elderly population. Physically, locomotion and daily activities become difficult, and vulnerability toward infections increases with aging.

OP is a skeletal disease characterized by a reduction in bone mineral density, predisposing people to an increased risk of fracture. The hypothesis that OP is an age-associated disease and mainly a consequence of estrogen deficiency was proposed in 1941.[1] In 1998, the "unitary model of OP in postmenopausal women and aging men" was put forward, this concept combined postmenopausal (involving mainly trabecular bone) and senile OP affecting both cortical and trabecular bone.[2] The management of OP involves various pharmacological options that can be divided into antiresorptive and bone stimulatory agents (the former are more broadly used in clinical practice). On the other hand, OA is a degenerative joint disease also associated with age that includes a group of pathologies of joint structures resulting in pain and disability. There is no disease-modifying treatment for OA, and the management of patients is currently limited to pain reduction and lifestyle modification. Increase in age is one of the risk factors for OA.[3] However, it is now

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recognized that aging and OA are independent processes, so that age is one of many contributory factors. Because OA has been historically described as a disease of the cartilage, most of the research on aging in OA has been focused on cartilage tissue.[4] More recently, OA has been proposed as a disease of the whole joint, which is additionally characterized by severe alterations to subchondral bone as well as by low-grade systemic and local inflammation.[5] Abnormalities in OA bone include subchondral plate sclerosis, bone marrow (BM) lesions, and the formation of osteophytes, all are a result of intermittent abnormal subchondral bone remodeling, which links this aspect of OA to the OP.[6]

One of the key cellular players in bone physiology is the osteoblast, a bone-forming cell derived from BM resident mesenchymal stromal cells (MSCs). BM-MSCs are self-renewing *in vivo,* and the shift in their differentiation from osteogenic to adipogenic lineage with increase in age[7–10] can potentially affect osteoblast formation and bone remodeling, in general, and thus be a factor in OP and OA development and pathogenesis. *In vitro* expanded MSCs have broadly been defined by their adherence to plastic, ability to give rise to at least 3 cell lineages (adipogenic, osteogenic, and chondrogenic); presence of CD73, CD90, and CD105 surface molecules; and absence of CD11b or CD14, CD19 or CD79a, CD34, CD45, and human leukocyte antigen—antigen D related (human leukocyte antigen—antigen D related [HLA-DR])—as defined by the International Society for Cellular Therapy.[11] However, the markers used for characterizing *in vitro* MSCs are not very useful for the identification of MSCs or their subpopulations *in vivo*.[12] Most of the experiments for understanding MSC aging have been performed using cultured MSCs, and attempts have been made to correlate the findings with *in vivo* environment. This article addresses the comparison of *in vitro* MSC aging with *in vivo* MSC aging and discusses the concept of *in vivo* MSC "rejuvenation" and its future prospects for novel therapies for OP and OA.

General Theories of Aging

In an attempt to understand MSC aging, it is worth reflecting on the general theories of cellular and whole body aging. Among many existing theories, DNA damage, the free radical (FR), telomere shortening, and stem cell theories have been discussed in detail recently.[13]

The DNA damage theory is among the earliest theories focusing on the mechanism of aging. It refers to the accumulation of DNA damage in every cell with the passage of time.[14,15] The sources of DNA damage vary from endogenous toxic chemicals and reactive oxidative species (ROS) to exogenous ultraviolet light. This theory has been explored to describe *in vitro* MSC aging, particularly in relation to the accumulation of chromosomal instability following prolonged culture.[16]

The FR theory or the theory of ROS proposes that the continuous process of FR generation in metabolism causes damages to tissues owing to the presence of free and unpaired electron on the oxygen atomic outer shell.[17] Antioxidants in the body combat the negative effects of ROS. However, when the balance between production of antioxidants and FR is lost, oxidative stress (OS) develops.[18] This can then lead to oxidation of nucleic acids, severely damaging the DNA and resulting in mutagenesis and modification of the transcription of specific genes. In terms of MSC aging, this theory has primarily been applied to describe the decrease in adhesion of MSCs[19] and the increased bias toward adipogenic differentiation.[20]

The telomere shortening theory postulates that with each cell division, the length of telomeres in mature cells keeps reducing due to the attrition and the lack of telomerase activity, an enzyme responsible for telomere elongation. In BM-MSCs, telomere attrition can also occur leading to cellular senescence,[21] although its exact mechanism remains unclear, as in MSCs, telomerase activity is present but low. While telomere length decreases with an individual's age, recent data suggest that after the age of 75, it becomes positively correlated with age implying its significance for survival in the very old age.[15,22] In terms of MSC aging, this theory has been extensively tested using serially passaged MSCs, where an average 17 base pair telomere loss with each population doubling was confirmed.[21,23]

The stem cell theory of aging postulates a decline in stem cell number and functionalities as a potential effect of aging.[24–26] Schultz and Sinclair have discussed that the probable causes of cellular aging (i.e., the abovementioned telomere attrition, DNA damage, and cellular senescence) can also be applied to stem cells.[15] Fukada et al. have not only described the aging of stem cells but have also connected the other theories with the stem cell theory to explain the complex progression of aging.[18] As BM-MSCs can be viewed as true stem cells,[27] the stem cell theory is linked to each of the theories mentioned above.

Finally, the theories of epigenetic alterations[24] and decline in protein homeostasis[25] have also gained importance in the recent past. While all these theories have been mentioned separately, they are interlinked and lead to one another. Mitochondrial damage often leads to the production of ROS that interact with molecules in the body affecting them negatively.[24] The accumulation of the oxidative by-products of ROS interactions, with time, causes DNA damage and genomic instability resulting in cellular senescence. This disturbs the self-renewal capacity of stem cells in their microenvironment which is known to interrupt the normal functioning of different types of stem and other cells in the BM.[18,28]

In Vitro MSC Aging

This section outlines the recent findings of *in vitro* MSC aging as well as the current methods used to track MSC aging in culture.

Passage Dependent

It is well accepted that MSC expansion in culture results in their accelerated aging.[23,29] Colony-forming unit fibroblast

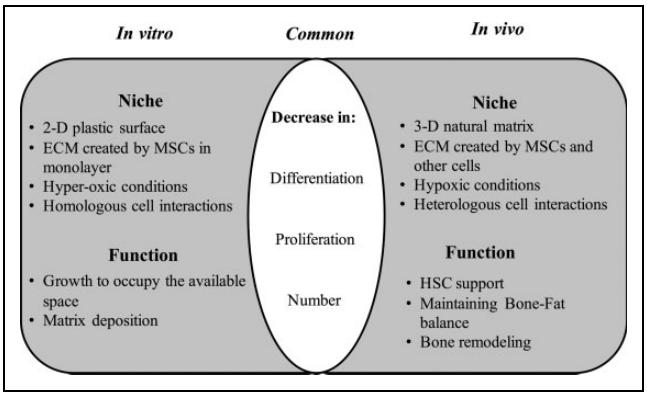

Figure 1. A proposed model highlighting the different mechanisms of bone marrow–mesenchymal stromal cells (MSCs) aging *in vitro* and *in vivo*. 2-D = 2-dimensional; 3-D = 3-dimensional; ECM = extracellular matrix; HSC = hematopoietic stem cells.

assay has been one of the oldest methods to document the loss of proliferation in cultured MSCs during their extended passaging.[30] Many independent studies have later documented that this loss of proliferation is correlated with a decline in the telomere length with increasing population doublings (PD).[23,29] The senescence-associated β-galactosidase (*SA-β-gal*) enzyme activity is increased in senescent cells and similarly, the number of *SA-β-gal* positive cells have been reported to escalate with increase in PDs.[29,31] Regarding the potentials of MSC for differentiation, a study by Vacanti et al. was among the first to show a significant decrease in the differentiation potential of cultured BM-MSCs (porcine) into osteogenic lineage in the late passages as compared to early passages. The late passages also exhibited actin accumulation, reduced adherence to substrates, and increased activity of β-galactosidase all of which are indicative of cellular aging.[32] More recently, Yao et al. have highlighted the effects of *in vitro* aging on wound healing ability of MSCs derived from mouse fat pads.[33]

In addition to the abovementioned changes, Bonab et al. observed anomalies in the morphology of human BM-MSCs with increasing passage number and suggested that *in vitro* aging of MSCs begins from the minute they are plated on plastic.[23] One of the potential reasons for such accelerated aging could be the fact that MSCs placed in culture receive conditions supportive of their enhanced proliferation that goes far beyond its physiological demands (Fig. 1). Importantly, MSCs are cultured under hyperoxia, where they consume oxygen at a high rate,[34] leading to high levels of ROS that accumulate with prolonged culture of MSCs.[35]

One of the suggested solutions to such accelerated aging could involve growing MSCs under hypoxic conditions. Under long-term exposure to hypoxia, MSCs may adapt themselves to low oxygen levels, decreasing their oxygen consumption and reducing their ROS production.[23] This would lead to improved MSC survival and reduced apoptosis. Interestingly, the migration of adipose tissue MSCs may also be induced by hypoxia as shown in animals.[36]

Donor Age Dependent

The closest to understanding *in vivo* aging of BM-MSCs in humans has so far been obtained from the studies where the growth and differentiation of MSCs from young and old donors have been compared using the same growth conditions *in vitro*. For example, Mueller and Glowacki observed a decline in the osteogenic differentiation potential of MSCs from human femoral BM collected from subjects above the age of 60 (old) compared to subjects below the age of 50.[8] Stenderup et al. studied the maximal *in vitro* life span and *in vivo* bone formation in mice of human BM-MSCs from young (18–29 years old) and old (68–81 years old) individuals. Their results suggested that the life span in cumulative PDs from the older donors was significantly lower than that of the younger donors and that the MSCs from the older donors exhibited accelerated senescence with each PD. However, they did not find any change in the total number of senescent cells (using *SA-β-gal* assay) or in the telomere lengths in the early passage cells of both groups of donors. Interestingly, MSCs from both the donor groups were able to form similar amounts of mineralized matrix and *in vivo* bone formation in mice. This provided the first indication that MSC aging *in vitro* possibly occurs faster than *in vivo* MSC aging.[29]

Baxter et al. measured the mean telomere restriction fragment at exactly the same "culture age" (16 PDs) of human BM-MSCs and found a significant decrease in the length of telomeres in the MSCs of older donors (59–75 years old) compared to the younger ones (0–18 years old).[21] Stolzing et al. measured ROS and superoxide dismutase levels to compare these and other indices of *in vitro* aging in BM-MSC cultures from younger and older donors. They observed an increase in all tested indices of aging in cultured MSCs from older donors, indicating a reduction in their "fitness."[31] Peffers et al. have used protein analysis of human MSCs from young and old donors and have discovered alterations in energy metabolism in older donors.[37] These studies suggested that BM-MSCs most probably "age" *in vivo,* but the use of cultured MSCs, which themselves undergo rapid "aging" *in vitro*, is not an optimal material to understand *in vivo* BM-MSC aging and other approaches need to be developed.

Methods to Track MSC Aging in Culture

Tracking *in vitro* aging of MSCs has been performed using a variety of methods, some of which have been mentioned already. One of the most intriguing observations noted in many studies pertains to considerable changes in MSC size and morphology. Initial passages of cultured MSCs retain their characteristic spindle-like morphology, but the cells after a few PDs appear enlarged and more granular.[23,38,39] The relative area of BM-MSCs in the late passages increases over 10-fold compared to early passages (from 5 μm^2 up to 50 μm^2, respectively).[40] This increase in size appears to

parallel with the increase in actin stress filaments.[29] A similar increase in size and actin filaments in late passage cells has also been observed in late passage of cultured osteoblasts.[41] While the reasons for these morphological changes in MSCs remain unknown, precise measurements of MSC size could provide a rapid estimate of an MSC aging "status" during their culture *in vitro*.

Immunophenotyping using several antibodies have been performed to identify surface markers specific for aged MSCs. An increase in the expression of CD44 and a decrease of Stro-1 molecule, CD71, CD90, CD105, CD146, and CD274 were detected in human BM-MSCs with increasing PDs as well as in old donors.[31,42,43] However, measuring surface markers as indicators of MSC aging remains controversial; for example, while some studies have observed a decrease in CD106 expression during MSC passaging,[44] others documented its gradual increase,[45] which is most likely due to the different culture conditions used. Gene expression and DNA methylation marks may, in this respect, be more useful.[39,46] For example, the analysis by Wagner et al. revealed over 1,000 transcripts upregulated at least 2-fold in senescent MSCs and over 500 transcripts downregulated.[39] More recently, Peffers et al. have shown that the expression of miR-199b-5p was reduced in MSCs from old donors and correlated with a decline in energy metabolism and cell survival.[37] Duscher et al. worked on murine adipose–derived MSCs and observed a decline with age in hypoxic transcription factor (*Hif1a*) and C-X-C motif chemokine ligand 12 (*CXCL12*) highlighting their impaired therapeutic potential.[36]

In Vivo MSC Aging

In vivo tracking of MSC aging has so far been performed only in animal models. While the available literature broadly suggests a decline in MSC frequency with aging,[47,48] the issue remains controversial. For example, some studies have indicated a decline in MSC number in older individuals,[31,49] whereas other scientists did not find any significant changes.[43,50] This could be due to different volumes of BM aspirate used as well as different processing methodologies (e.g., direct plating vs. density centrifugation).[51,52] One of the most interesting features of MSCs from older individuals appears to be their reduced propensity for osteogenic differentiation with increased bias toward adipogenic differentiation. The loss of balance between osteogenic–adipogenic differentiation leads to increased BM adiposity and is seen in OP.[7,10,28,53] The exact mechanism underlying the adipogenic bias is yet to be clearly understood. Recent studies have identified microRNAs miR-27a,[54] miR-27b, Let-7G, and miR-106a[55] that are necessary for maintaining the osteogenic differentiation potential of MSCs and have displayed a significant decline with aging.

As mentioned in the above sections, the great majority of studies investigating the aging of human BM-MSCs have been performed on MSCs expanded in culture. As these cultured MSCs undergo an aging process as a result of extensive proliferation during consecutive PDs, these studies provide only indirect evidence of how MSCs may age *in vivo*. Furthermore, *in vivo* BM-MSC niche is not mirrored when these cells are cultured and expanded on the plastic surfaces. Thus, it can be proposed that *in vivo* and *in vitro* aging of MSCs may have both common (overlapping) and nonoverlapping features (Fig. 1).

Intrinsic Factors: Proliferation Burden

In vitro MSC aging could, to a large extent, be due to telomere erosion occurring as a result of their rapid proliferation in response to growth factors (GFs) present in fetal calf serum. MSCs grown in medium supplemented with human platelet lysates grow even faster.[56] As shown for fibroblasts,[57] the chronic exposure of MSCs to high doses of growth promoting factors could lead to the downregulation of GF receptors and resistance to these factors and eventually resulting in cellular senescence.

In contrast, there is no compelling evidence that MSCs *in vivo* are rapidly cycling cells; in the opposite, earlier studies have documented that the *in vivo* MSCs identified based on Stro-1 expression are slow cycling.[58] *In vivo* MSC proliferation, and factors that influenced it, is not fully understood, but the fact that human bone constantly undergoes a process of remodeling so that every 10 years mature bone cells are renewed[59] suggests that *in vivo* MSCs should undergo rare asymmetrical divisions in order to both maintain their own pool and also to provide enough osteoblast progenitors to facilitate bone remodeling. Because of this slow-cycling nature of *in vivo* MSCs, their aging due to telomere erosion is unlikely to be the primary factor in their *in vivo* aging process. It is noteworthy that following bone fracture, the local MSC proliferation response is activated[60]; furthermore, local injections of platelet-derived factors can temporarily increase their local pool.[61] There is some evidence that blood platelet responses following fracture may also exert a systemic effect and "activate" these slow-cycling MSCs.[62]

Environmental Factors: Stem Cell Niche

The stem cell niche is commonly defined as an *in vivo* regulatory microenvironment where stem cells reside.[63] MSC niches in human BM are poorly understood but are believed to be primarily in the perivascular and bone-lining locations.[64] Notably, cells expressing a common BM-MSC marker LNGFR/CD271 may be more broadly distributed as adventitial reticular cells, which are connected to each other via long projections, forming the "backbone" of the BM stroma.[65] These topographical differences should be taken into account when considering how BM-MSCs may age *in vivo*. For example, perivascular BM regions are believed to be more oxygenated compared to endosteal regions[66] and so perivascular MSCs could theoretically be more exposed to OS compared to bone-lining MSCs. However, recent direct

in vivo measurements of local oxygen tension in the BM of live mice showed perisinusoidal rather than endosteal regions to be more hypoxic suggesting that the BM hypoxic landscape is determined not only by vascularity (oxygen supply) but also by the area's cellularity (consumption).[67]

As the MSC niche in BM generally has low oxygen level, they must be adapted to use mainly an anaerobic metabolism (glycolysis) for their energy supply, which appears to limit the MSC proliferation to avoid OS.[35] A phenomenon called extra physiological oxygen shock/stress (EPHOSS) was first noted for hematopoietic stem cells (HSCs) when BM was collected and processed in ambient air (21% oxygen).[34] The EPHOSS is associated with loss of stemness and is related to increased level of mitochondrial ROS.[34] Although the level of ROS is induced during *in vitro* MSC aging, this event has been reported at similar levels between young and old animals (rats).[68] It was proposed that the increase of ROS was more related to microenvironment of culture than chronological age.

Environmental Factors: Effect of Hormones and GFs

The significant drop of sex hormone levels and the increase of the glucocorticoids production and activity are hormonal hallmarks of human aging that are associated with reduced bone mass.[69,70] The functional capacities of MSCs have been linked to these age-related hormones. Estrogen can induce osteogenic rather than adipogenic differentiation of MSCs.[71] Conversely, testosterone promotes the proliferation of MSCs and preserves their stemness.[72] This may explain a more prominent decline in *in vivo* MSC numbers in females compared to males.[43,73] The *in vivo* effect of glucocorticoids on MSCs is not clear, but these hormones have a negative effect on osteogenesis as inducer of the apoptosis in osteoblasts and suppressor of their differentiation and proliferation.[74] In contrast to the *in vivo* hormones, the hormones incorporated into the MSC expansion or differentiation medium could have different effects. For example, very high doses of glucocorticoids added to MSC differentiation media stimulate their differentiation into fat, cartilage, bone, and muscle cells.[75]

An interesting GF that can be implicated in *in vivo* MSC aging is insulin-like growth factor 1 (IGF-1). The blood and bone matrix levels of IGF-1 are decreased significantly with aging and correlated with reduced bone mineral density.[76] The osteogenic differentiation potential of MSCs is enhanced by the effect of IGF-1 as shown in mouse IGF-1 model of receptor knockout.[77] Thus, it could be assumed that the reduction in IGF-1 effect on MSCs is another characteristic of their *in vivo* aging.

Environmental Factors: The Cross Talk with Other BM Cells

The cross talk between HSCs and MSCs within BM is a dynamic process that affects the functions of both cells and probably their aging as well. Within their common niche,[67] HSCs also undergo alterations with aging. Studies in animals have confirmed the process called "myeloid skewing," which includes a decline in the number of lymphoid progenitors and a bias toward the formation of myeloid progenitors with increasing age.[78]

It is quite likely that both types of stem cells are influenced by similar "aging forces." In fact, some studies have indeed shown that BM-HSC and MSC aging occur in parallel.[50] Interestingly, MSCs can help HSCs to reduce their intracellular ROS levels via several mechanisms including C-X-C chemokine receptor type 4 (CXCR4)/CXCL12 (stromal cell-derived factor 1 [SDF-1]) interactions and an uptake of ROS via connexin gap junction.[57] While there has been more focus on the niche-related mechanisms that might induce aging of HSCs (changes in connexin gap junction and role of SDF-1), it is still unclear whether these changes in the *in vivo* niche/milieu could also affect the aging of MSCs. It will be valuable to determine to what extent the surrounding cells (e.g., HSCs) would affect the *in vivo* MSC aging.

Mature MSC and HSC descendants and the molecules they release may also play a significant role in MSC aging. For example, it is well known that marrow adiposity (i.e., the numbers of fat cells, which are mature MSC descendants) increase with age.[79] The factors that mature adipocytes release into their environment (adipokines and other adipose tissue hormones) are likely to influence the neighboring MSCs, potentially creating a "vicious loop" that drives preferential MSC differentiation toward fat rather than bone, which likely impacts also on the decline in hematopoiesis in this area and the propensity to OP.

Rejuvenation of OP and OA MSCs

Early studies have shown a decline in the number of osteoblast progenitors in the BM of OP patients compared with age-matched controls.[80] At least in females, this could be explained by an altered balance in the systemic levels of hormones such as estrogen and testosterone. Culture-expanded MSCs from OP patients have been shown to possess lower proliferative and osteogenic capacities[81] (i.e., to exhibit distinctive marks of premature *in vitro* aging) compared to healthy individuals. Recent evidence suggests that this could be due to the overexpression of osteogenic inhibitors in OP MSCs.[82] A recent study by Zhou et al. has identified a number of differentially expressed genes that are up- or downregulated in OP MSCs, which could not only be used as a biomarker for OP,[83] but also serve as potential targets for therapeutic modulation to rejuvenate OP MSCs. MSCs extracted from the areas of subchondral bone damage in hip OA patients also appear to acquire several abnormalities indicative of premature aging (e.g., reduced *in vitro* proliferative and mineralization capacities).[84] It remains to be investigated whether putative accelerated aging of OP and OA MSCs *in vitro* is reflective of their accelerated aging *in*

Table 1. Abnormalities in MSCs or Their *In Vivo* Niches as Potential Targets for OP and OA MSC Rejuvenation.

Abnormality	Intrinsic/Environmental	OP	OA
MSC number	Intrinsic	Reduced[80]	Unbalanced in damaged areas[84,86]
Signaling pathways in MSC	Intrinsic	Reduced osteogenesis due to overexpression of osteogenic inhibitors[82]	Abnormal homing due to altered TGFβ signaling[86] and chemokine receptor expression[84]
MSC niche: ECM	Environmental	Increased BM adiposity[53]	Increased BM adiposity[85] Increased levels of free TGFβ[86]
MSC niche: Neighboring cells	Environmental	Accelerated osteoclast activation[53]	Increased numbers of osteoclasts in damaged areas[86]

Note. BM = bone marrow; ECM = extracellular matrix; MSC = mesenchymal stromal cells; OA = osteoarthritis; OP = osteoporosis; TGFβ = transforming growth factor β.

vivo. However, as mentioned in the above sections, OP MSCs may indeed reside in a considerably modified *in vivo* niche that is markedly enriched in marrow fat.[53] Changes in marrow adiposity and an increased cellular necrosis have also been detected in the affected areas of OA subchondral bone.[85] This suggests that targeting putative *in vivo* aging in OP and OA MSCs may be performed indirectly, by targeting their *in vivo* niches (Table 1).

Abdallah et al. tested the impact of serum from young (20–30 years old) and old (70–84 years old) donors on MSC differentiation and proliferation and found a significant decline in osteogenic differentiation potential of MSCs grown in serum from old donors.[87] This suggests the existence of, yet unknown, factors in the serum or plasma from "younger" donors that could potentially be used systemically to reduce the rate of *in vivo* aging of MSCs, including OP MSCs. Similarly, Sun et al. cultured MSCs from young and old mice on plastic, young extracellular matrix (ECM) and old ECM and have shown that the number and quality of MSCs from old mice could be improved when cultured on young ECM.[88] *In vivo* ECM modulation could thus be another strategy to rescue or rejuvenate MSCs in OP and possibly, OA.

The "mechanistic target of rapamycin" (mTOR) is a serine/threonine protein kinase of the phosphatidylinositol-3-OH kinase (PI3K) family, which regulates cell growth, metabolism, and functions in two complexes—mTOR complex 1 and mTOR complex 2. This pathway has been the target of wide interest ever since it was discovered, and it has been suggested that inhibition of this pathway could extend the life span of rodents.[89] The role of rapamycin in the differentiation of MSCs or their progenitors has also been studied, but to date, the results are controversial,[90] suggesting that the drug can either encourage[91] or discourage[92] osteogenic differentiation of MSCs. This implies the need for further exploring this pathway and related molecules to understand their function in MSC aging and their possibility to rejuvenate aged MSCs.

Sirtuins (SIRT) genes have similarly proved to be of significant importance in regulating aging.[93] Simic et al. performed experiments on mice with MSC specific sirtuin 1 (Sirt-1) knock out gene and found that Sirt-1 regulates the MSC differentiation by the deacetylation of β-catenin.[94] This suggests that targeting this gene using gene therapy can be used for halting or reversing aging of MSCs. However, further studies are required to understand the connecting links between the SIRT genes and this signaling cascade to ultimately apply this approach for preventing aging of MSCs *in vivo* and in diseases such as OP and OA.

Conclusions and Future Directions

Currently, our understanding of MSC aging *in vitro* considerably surpasses our understanding of MSC aging *in vivo*. In order to move forward in the study of human MSC aging *in vivo*, an ability to isolate a pure population of uncultured MSCs represents an initial and critical step. Up to now, a broad consensus on the combination of markers to purify human BM-MSCs does not exist, although markers such as CD271 and MSCA-1,[12] possibly in combination with CD140a,[27] appear to be the most promising.

Methods currently used for the detection of aging in cultured MSCs could, in principle, be used for the study of aging of uncultured MSCs, although these need to be adapted to be used with very low numbers of cells as BM-MSCs are very rare *in vivo*. Research based on molecules like prelamin and lipofuscin, which can be[65] detected at the later stages of MSC culture *in vitro*, is a promising approach for identifying "aged" and senescent MSCs *in vivo*.[39,95] It will be interesting to investigate the change of size of BM-MSCs with aging *in vivo* and compare it to the acknowledged change of size *in vitro*[40] to possibly use cell size as an indicator of MSC aging *in vivo*. Churchman et al. have demonstrated a decline in the expression of molecules like connexin 43 in uncultured human BM-MSCs in older donors using quantitative polymerase chain reaction,[96] suggesting that it, and similar surface molecules, could be considered further as potential indicators of aged MSCs *in vivo.*

The ability to identify MSCs in situ and extract them less invasively from their native niches would then lead to a better understanding of their local environments, which could then be reconstructed and modeled using 3-dimensional (3-D) "organ" cultures. These organoids could then be used for developing and screening new

rejuvenation compounds to target OP and OA MSCs in 3-D environments most closely resembling their native niches.

These approaches should lead to a better understanding of MSC aging *in vivo*. Considering that MSCs form an integral part of the musculoskeletal system and that OP and OA have been associated with aging,[1,3] prevention of MSC aging *in vivo* could lead to novel therapies to target altered bone formation in OP and OA.

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