Age-associated changes in the ecological niche: implications for mesenchymal stem cell aging

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Abstract
Adult stem cells are critical for organ-specific regeneration and self-renewal with advancing age. The prospect of being able to reverse tissue-specific post-injury sequelae by harvesting, culturing and transplanting a patient’s own stem and progenitor cells is exciting. Mesenchymal stem cells have emerged as a reliable stem cell source for this treatment modality and are currently being tested in numerous ongoing clinical trials. Unfortunately, the fervor over mesenchymal stem cells is mitigated by several lines of evidence suggesting that their efficacy is limited by natural aging. This article discusses the mechanisms and manifestations of age-associated deficiencies in mesenchymal stem cell efficacy. A consideration of recent experimental findings suggests that the ecological niche might be responsible for mesenchymal stem cell aging.

Introduction
Experimentation with mesenchymal stem cells (MSCs) has long moved beyond the pre-clinical phase. MSCs are currently being tested in several ongoing clinical trials for cardiac muscle repair, bone regeneration and joint repair [1-8]. Stem cells are building and regenerative tools; they are fundamental to the body’s ability to self-renew with advancing age [9-15]. Exactly what criteria define the immortality of stem cell lines is variable and is dependent on the goals of individual investigators [16]. The longevity of ex vivo MSCs can be preserved by successively selecting for cell lines with the highest fidelity [16], but the same cannot be done in the whole organism. Time introduces selective and environmental constraints that diminish the fitness of adult stem cells [10-16]. It is increasingly clear that stem cells are subject to the same factors that introduce the genotypic and phenotypic changes associated with “wear and tear” in other somatic cells [10-12,16], but their robust ability to detect and resist damage, and continuously produce progeny with properties akin to parental cells sets them apart [10,11]. Of importance is the distinction between replicative and chronological aging [12,17-22]. Stem cells are highly proliferative; adult stem cells in particular have a finite replicative lifespan that is determined to a large degree by telomere attrition [11,19]. The growth arrest and resultant cellular senescence displayed after a specific number of population doublings alone [20] are not sufficient to completely compromise stem cell functionality in vivo. They do not correlate directly with the lifespan of the whole organism [21-24]. Ex vivo adult stem cells isolated from aged donors display characteristic features of both chronological and replicative aging. This is typified by the accumulation of damaged macromolecules, and cellular constituents crucial for efficient DNA replication and repair. Other characteristic features are stress-related genome instability, loss of function, and changes in patterns of immunophenotype marker, gene and protein expression [10-16,18-30].

Aging limits the therapeutic potential of mesenchymal stem cells
Quantitative and qualitative measures of MSC potency define the range of tissue-specific phenotypes into which they can differentiate. Their self-renewing and regenerative ability in vivo correlates directly with the extent of ex vivo proliferative and clonogenic ability. Ex vivo comparison of MSCs isolated from young and aged animals [27-30] and in vitro assessment of isolated MSCs over several population doublings [31,32] are the most utilized experimental aging models. These models are instructive in terms of delineating the extent to which MSCs are subject to the effects of natural aging, but they do not definitively reproduce events of natural aging.
in vivo [21-23]. Results from systems level analyses of this sort force us to confront the reality that MSCs, like other adult stem cells, do not escape the deleterious effects of natural aging. MSCs lose specific aspects of their appeal with natural aging. A key advantage with MSCs is their immediate availability in clinically relevant numbers for acute and long-term therapy. They can be isolated and expanded in vitro in a matter of days [8, 27, 29, 33], but advanced donor age correlates directly with a depleted MSC population [27-29]. This raises questions about their prompt availability in large numbers for autologous transplantation.

The basic but oversimplified understanding of cell therapy is that dead tissue can be repopulated by direct application of exogenous cells. The approach has therefore been two-pronged - direct administration of exogenous MSCs, and reliance on their homing ability to further stimulate endogenous repair. To create viable tissue, transplanted MSCs must survive, engraft and communicate with endogenous cells. Secondary to engraftment and electro-mechanical coupling is transdifferentiation into functional host cells. MSCs lack the level of pluripotency associated with embryonic stem cells (ESCs) but maintain robust clonogenicity and multipotency. They can give rise to adipocytes, chondrocytes, osteoblasts, and cardiomyogenic, neurogenic, and endothelial cells in vitro [8, 33-35] (Figure 1). Furthermore, the ability of MSCs to seek out injured tissue is cytokine mediated [29].

Multilineage differentiation, cytokine, paracrine, anti-apoptotic and angiogenic capacity is fundamentally age compromised in MSCs [27-30] (Figure 1). Asumda and Chase [27] demonstrated that MSCs derived from aged rats fail to express the adipocyte lipid-binding protein FABP4, osteocalcin, and aggregan following induction with adipogenic, osteogenic and chondrogenic media, respectively. In contrast, cells derived from young rats display extensive differentiation capacity by forming adipocytes, osteocytes, chondrocytes and cardiomyogenic cells following induction with differentiation media [27] (Figure 1). These specific differentiation and proliferation data from Asumda and Chase [27], further substantiated by Yu and colleagues [30], demonstrate a fundamental loss of function that, by extension, means MSCs lose the ability to respond effectively to injury [16, 35]. The issue of electro-mechanical coupling with endogenous cells is further explored by Asumda and Chase [27] under co-culture conditions. They showed that, in comparison to young MSCs, old MSCs express significantly low levels of the gap junction protein connexin-43 [27]. Dye coupling and positive double color staining experiments show that connexin-43 is required for cell-cell gap junctional communication and for electro-mechanical coupling between cardiomyocytes and MSCs [27, 36-39]. Despite expressing low levels of connexin-43, old MSCs fused with adjacent ventricular cardiomyocytes and expressed GATA 4, Nkx2.5, cTnI, cTnT and myosin heavy chain [27]. The point must be made here that these co-culture experiments [27] did not investigate functional coupling between MSCs and ventricular cardiomyocytes. Asumda and Chase [27] did not conduct electrophysiological studies, and MSCs were not uncoupled from ventricular myocytes for further determination of the cardiac phenotype. It is not clear, therefore, that aged MSCs acquire an increased differentiation capacity following direct contact with cardiac myocytes. In the absence of this type of assessment, we cannot definitively distinguish stem cell plasticity in terms of transdifferentiation on the part of co-cultured old MSCs as opposed to cell fusion with cardiomyocytes [39, 40]. The expression of cardiac specific markers in the co-cultured aged MSCs [27] is, in all probability, the result of cell fusion that is the formation of hybrid cells with simultaneous expression of donor and recipient cell markers [39, 40]. The implication here is that cells isolated from older individuals for therapy might be far too impaired to actively transdifferentiate into contractile cardiac myocytes or organ-specific cells.

In comparison to other candidate stem cells, MSCs possess superior genetic stability. The risk of their spontaneous transformation to cause oncologic issues following transplantation is minimal [41-43]. It is important to take into account the slight interspecies variations. Murine MSCs are fundamentally more genetically unstable when compared to human MSCs [42, 44-46] and are significantly more likely to cause oncologic issues. Data from non-human cells can therefore not be extrapolated definitively to humans [46, 47]. Age-associated genomic instability is implicated in the spontaneous malignant transformation of ex vivo MSCs [44-49]. So while the evidence for malignant transformation of human MSCs in clinical trials is fiercely questioned and is murky at best [41-43, 50-59], the deleterious effects of aging [50] nonetheless present a serious risk factor for in vitro transformation and ectopic tissue formation following transplantation.

MSCs are immune privileged and immunosuppressive; surface immune antigens are present at minimal levels [8, 59, 60]. This unique immunophenotype gives them a selective advantage and is fundamental to their appeal in the clinical setting. T-lymphocyte proliferation is suppressed, immunogenic MHC-Ia expression is marginal, and immunosuppressive MHC-Ib is upregulated [8, 60-63]. The effect of natural aging on MSC immunogenicity has not been studied directly and extensively. It is not known, therefore, if MSCs lose their immune privilege properties with advancing age. A consideration of recent experimental findings suggests that MSCs are not intrinsically immunoprivileged [63, 64]; they are immunogenic in
immunocompromised animals [64,65]. This suggests that MSCs require a supportive microenvironment - one with a set and minimal number of factors - to effectively exert their immunoregulatory effects on immune cells [63]. Whether natural aging exacerbates MSC immunogenicity is an open-ended question. It is presumed here that the deleterious effect of aging on the micro-environment will have dire consequences for MSC immune regulation following transplantation. In line with this thinking, the distinction should be made between presumptively compromised allogeneic ex vivo MSCs isolated from aged donors that are inherently immunogenic prior to transplantation, and ex vivo MSCs that either fail to suppress immune cells, and/or elicit an immune response following transplantation due to an age-compromised host microenvironment.

Figure 1 Representative micrographs depicting morphological change after 21 days of exposure to differentiation media. Representative micrographs show anti-FABP4, anti-osteocalcin, anti-aggreca and anti-cardiac myosin heavy chain staining in differentiating bone marrow-derived mesenchymal stem cells (BM-MSCs). BM-MSCs derived from aged rats fail to undergo adipogenic, chondrogenic, osteogenic and cardiomyogenic differentiation. BM-MSCs derived from young rats transform into fat-forming adipocytes, cartilage-forming chondrocytes, bone-forming osteocytes and cardiomyogenic cells. BM-MSCs were isolated from ‘aged’ and ‘young’ Sprague Dawley rats (15 months and 4 months).
Despite the lack of data in direct support of age-associated intrinsically immunogenic MSCs, the idea is not entirely anecdotal. Li and colleagues [66] recently demonstrated that extensively passaged MSCs, in addition to displaying characteristic senescence-associated aberrations, lose their immunosuppressive effect on T-cell proliferation. Additionally, MSCs express and rely on toll-like receptors (TLRs) along with Notch signaling to exert T-cell immunomodulatory effects [67]. TLR function [57] and Notch expression [68] are diminished in aged humans. Furthermore, the dysregulation of Notch signaling is associated with the aging phenotype in stem cells [69]. The ultimate test for MSCs in the clinical setting will be their ability to improve quality of life and extend the life of patients; however, for the foreseeable future, the fact that MSCs do not escape the adverse effects of natural aging will be a major limitation for their use in cell therapy.

**The ecological niche and mesenchymal stem cell aging**

From a clinical and translational stand point, the aging issue raises a number of questions. The key question is if MSCs from elderly donors fundamentally provide the same healing effect as their younger counterparts? At present, no clinical trial has explicitly tested this
hypothesis so the answer is not straightforward. A look at past and ongoing phase I to III clinical trials testing the efficacy of MSCs shows an upper age limit up to 90 years [9]. Ex vivo MSCs must be expanded over several population doublings prior to treatment. Extensive passaging diminishes cytoskeleton turnover, and compromises mitochondrial morphology. It also impairs antioxidant capacity, and increases susceptibility towards senescence [70,71]. Geißler and colleagues [70] established that serial in vitro passaging of both young and old MSCs produces molecular alterations independent of chronological in vivo aging. Their data demonstrate that, in addition to the fundamental loss of differentiation capacity, MSC morphology, migration potential, and mitochondrial and cytoskeletal function is impaired over several population doublings [70]. This raises questions about the quality and potency of extensively passaged old MSCs as a therapeutic agent. The issue of donor age can be overcome with allogeneic cells but it presents a potential limitation for autologous therapy [35]. The underlying mechanistic question is if MSCs age intrinsically irrespective of the microenvironment or if the microenvironment is responsible for the aging phenotype? An extension of that question is whether MSCs are genetically programmed [16] like other somatic cells to wilt with time? Or are the observed alterations [27] simply a reaction of the cells to deleterious conditions that arise in their immediate microenvironment [16]?

For the purposes of simplicity, the bone marrow (BM) stem cell niche is examined in this review. Primary explant MSCs isolated from an aged BM compartment at low passage display defects [27] consistent with both replicative and chronological aging: this implicates the niche environment (Figure 2). Specific evidence for this line of thinking is sourced in part from Assmus and colleagues [72]. Their data show that significant changes occur in the BM stem cell niche following acute myocardial infarction that directly affect BM mononuclear cell function [72]. Cells resident in the BM compartment are sensitive and responsive to changes in their niche [72-76]. The niche is in turn responsive to changes in the global systemic milieu [16,77-79]. It is therefore understood that ecological interactions of the BM niche are critical to resident stem cell function [80]. By the same token, the aged tissue microenvironment to which exogenous stem cells are transplanted presents an inhibitory effect [81,82]. So while aged ex vivo MSCs might function in a cell autonomous fashion in culture, their interactions with the niche and the microenvironment that they encounter post-transplantation is bidirectional. Alterations in the niche as a consequence of natural aging will, in all probability, affect the survival and integration of transplanted cells.

The dynamic nature of MSC interactions with healthy and injured host cells in the aging microenvironment will, for example, influence their mitotic and differentiation ability. What is not clear at this stage is the degree to which intrinsic factors drive the aging process in MSCs independent of a changing microenvironment. No single factor has been shown to be responsible for global MSC aging. All the evidence points to the MSC niche environment and the effect that alterations within that micro-milieu has on the cells. This might explain why ex vivo cells from aged donors are compromised at primary isolation [27]; the key seems to lie in prevention. Presumably, the solution is to recreate a microenvironment that is identical to that of young patients. What remains to be determined is factor(s) that can be manipulated to alter the MSC-specific stem cell niche. Perhaps we can learn from studies conducted on non-MSC stem cells. Using the Drosophila testis stem cell niche, Toledano and colleagues [83] demonstrated that increased expression of let-7 accelerates the aging of stem cells by decreasing Imp levels in Drosophila niche cells. Imp regulates upd, which encodes a self-renewal factor; Upd promotes the stemness of adjacent Drosophila niche cells via activation of JAK-STAT signaling [84]. RNA interference-mediated inhibition of Imp in the niche results in reduced Upd levels [83]. The downstream effect of low Upd levels is a diminished number of germ line stem cells in the niche. By specifically targeting Imp expression, Toledano and colleagues [83] were able to rescue the age-associated loss of germ line stem cells. The implication of these results for MSC aging is clear - it suggests that the niche does in fact lose its supportive role with advancing age irrespective of the specific stem cell type. The take-home message here is that we might be able to rescue the age-associated loss in plasticity, and decrease in MSC population. This can be achieved either by preventing the expression of specific factor(s) or blocking the destruction of others [83].

The argument for targeting the micro-milieu is further strengthened by data from Conboy and colleagues [82] and Boyle and colleagues [84]. By exposing satellite cells from old mice to young serum containing specific factors, Conboy and colleagues [82] were able to rescue Notch expression and enhance proliferation in vitro. A translation of these findings to MSCs will suggest that we can reverse the age-associated changes by manipulating the systematic milieu to either increase the presence of anti-aging factors associated with young serum or decrease the levels of deleterious factors associated with aged serum. The details are still being worked out for MSCs and the BM stem cell niche. We do not have a clear picture of definitive MSC-specific anti- or pro-aging factors to enable the level of mechanistic studies described for muscle and Drosophila stem cells. The
good news is that most of the signaling factors and pathways already studied in other stem cell types overlap between cells. What we do know is that the BM stem cell niche [72-76] functions as a nest environment. It buttresses MSC viability, stemness, activation, migration, and overall function and protects against cumulative genetic damage [72-76]. The available data suggest that it undergoes fundamental deleterious changes with natural aging that work to diminish MSC function and self-renewal (Figure 2).

Another recent report by Conboy and colleagues [85] put forward data that demonstrate that ESCs secrete pro-regenerative anti-aging factors that counter the deleterious effects of the aged niche. This piece of information is significant for MSCs because Oct-4+, SSEA-1+, Sca-1+, Lin-CD45- very small embryonic-like stem cells (VSELs) within the BM are age depleted [86,87]. The specific role of VSELs in aging is not well studied; it is presumed here that age-associated changes in VSEL function are consequential for the BM stem cell niche. Their developmental origin [86-89], and the fact that a supportive BM microenvironment is required for their continued fidelity [89], suggests that VSELs are a key contributory component of the BM-derived MSC niche. It has been suggested that VSELs associate with, and play a contributory role to, MSC plasticity [88,89]. MSCs are a heterogeneous cell population; their expression of embryonic and pluripotency-associated markers is suggestive of prior contamination by VSELs during primary isolation [88]. Hence, the age-associated collective loss of pluripotency-associated genes by MSCs and other BM stem cells, such as VSELs, multipotent adult progenitor cells and marrow isolated multilineage inducible (MIAMI) cells [88], is suggestive of molecular level sensitivity to aberrant changes in the niche. This line of thinking is consistent with observations made by Asumda and Chase [27] and substantiated by Yew and colleagues [32]. Data from both studies show that ex vivo MSCs from aged rodents [27] and humans [32] show alterations in their expression of stemness and pluripotency-associated genes.

**Modifying and targeting specific factors in the niche**

In thinking about what specific anti- or pro-aging factors to target within the BM stem cell niche, the studies by Asumda and Chase [27] and Yew and colleagues [32] are a starting point. The expression of stemness and pluripotency genes is critical for ESC and adult stem self-renewal [75,90,91]. OCT4, SOX2, Rex-1, leukemia inhibitory factor, and fibroblast growth factor are implicated in the maintenance of MSC stemness [75,92]. These are also anti-aging factors that promote MSC function but are age diminished in MSCs [27,32]. The connection between changing conditions within the BM micro-milieu and the inhibition of these factors is not clear. Polycomb complex proteins are known regulators of OCT4, SOX2, and NANOG [75,93,94]. It has been suggested that Polycomb proteins might be involved in the activation and maintenance of OCT4, SOX2, Rex-1 and NANOG in MSCs [75]. Mechanistic studies to determine the association between self-renewal and stemness genes, the BM aging micro-milieu and the aging phenotype in MSCs are justified. Recent data from Han and colleagues [95], for example, show that age-associated loss of cardiomyogenic differentiation and proliferation ability in MSCs is reversible via forced expression of NANOG alone. They also demonstrate that forced expression of Nanog alone restores transforming growth factor (TGF)-β and p53/p21 signaling in ex vivo aged MSCs [95].

MSCs residing in the BM stem cell niche interact with stem and non-stem cells. They are responsive to the local extracellular matrix and to a host of paracrine and trophic factors [13,75,80] secreted in the local milieu. Notch, TGF-β, mitogen-activated protein kinase (MAPK) [73,75] and Wnt [63,71,84] signaling are implicated in stem cell aging and specifically in the BM stem cell niche [15,73,75,76]. Wnt3a promotes MSC self-renewal by increasing the pool of mesenchymal progenitors, enhancing proliferation and maintaining the undifferentiated state [96]. The drawback to Wnts is that their effect is pleiotropic - in the presence of Wnt3a, MSC osteogenesis is suppressed but Wnt5a promotes alkaline phosphatase expression in MSCs undergoing osteogenesis [96]. The complexity of Wnt protein signaling precludes an outright identification of a single mechanism that links alterations in the BM niche with Wnt and MSC aging. Boland and colleagues [96] postulate that specific spatial or temporal regulation of Wnt signaling and crosstalk with other pathways might boost the progenitor cell pool within which MSCs reside. In their model, canonical Wnts (Wnt3a and Wnt9a) function to maintain the undifferentiated proliferating pool of progenitor BM mononuclear cells [96]. On the other hand, non-canonical Wnts (Wnt5a and Wnt11), which are implicated in MSC osteogenesis and chondrogenesis, will presumably enhance MSC plasticity [96]. This proposed model of in vivo MSC maintenance and differentiation by Wnts has implications for the BM stem cell niche and MSC aging. Zhang and colleagues [97] demonstrated that exposure of MSCs to old rat serum increases reactive oxygen species production and expression of growth inhibitory and pro-aging factors such as p16INK4a, p53/p21 and γ-H2A.X via the activation of Wnt/β-catenin signaling. Zhang and colleagues [98] again showed that Wnt/β-catenin signaling directly induces MSC aging by activating reactive oxygen species
generation in MSCs. It is presumed here that such specific age-associated alterations as a consequence of changes in the ecological niche environment will have consequences [27] (Figure 2) for MSC function and self-renewal.

Notch-1 expression is diminished in ex vivo MSCs isolated from aged patients [99]. Direct inhibition of Notch-1 results in decreased bone formation [99]; this is suggestive of an age-associated loss in responsiveness to Notch-1 inducers [69]. Cell-cell interactions are crucial for MSC function in the BM stem cell niche [73-76]. Notch receptors are expressed on MSCs, along with their ligands [100]. Notch signaling is highly conserved and controls diverse cellular processes [100]. The link between faulty Notch signaling and aging in stem cells is well established [10,68,69,82,85]. Proximity between MSCs and stem, and non-stem cells within the three-dimensional BM micro-milieu affect MSC function and self-renewal. The Notch receptor is activated primarily via direct cell-cell contact and is critical for cell-cell communication [100]. Phenotypic and developmental changes in cell proliferation, differentiation and apoptosis are communicated between neighboring cells via Notch signaling. Presumably, age-associated changes in the niche environment will have consequences for Notch signaling and subsequent MSC activity. Studies conducted by Conboy and colleagues [101] show that forced activation of Notch rescues the loss in regenerative potential seen in old muscle. MSCs express all three Notch ligand receptors [100]; it is therefore a legitimate target for reversing age-associated changes in MSC efficacy.

The DNA damage model of aging postulates that aging is a direct result of long-term accumulation of deleterious alterations in DNA structure. The link between DNA damage and aging is well established; MSCs are not exempt in terms of deficient DNA repair. Asumda and Chase [27] demonstrated increased DNA damage and lowered telomerase activity as well as altered expression of stemness markers in old MSCs [27]. A similar set of observations were made by Yew and colleagues [32]. BM from older animals was slow to yield plastic-adherent MSC colonies. The resultant old MSCs had a spread out, flat, enlarged morphology with nuclei that appear larger than normal [27]. This phenotype is reminiscent of extensively passaged MSCs [27]; Yu and colleagues [30] made similar observations. The former set of observations (DNA damage, stemness genes, telomerase activity) is an aspect of intrinsic aging. This is otherwise unavoidable in non-stem tissue cells due to increasing, and cumulative use of specific signaling pathways over time [82]. The latter set of observations (morphology) is characteristic of physical and molecular level deterioration in response to adverse external changes. Asumda and Chase [27] assessed MSCs at low passage immediately following primary isolation. When taken together, these data indicate that specific age-associated cell-extrinsic changes occur in the BM compartment that directly influence component cells by altering cell-intrinsic factors [16] (Figure 2). Presumably, deleterious changes that occur in the stem cell milieu under pathological conditions should accelerate the aging process in chronologically young individuals so that the same defects observed in cells derived from aged donors are evident in cells derived from young but diseased donors [103,104]. For example, the differentiation capacity and efficacy of MSCs derived from donors suffering from Hutchinson-Gilford progeria syndrome (a premature aging disease in which uncharacteristically accelerated aging is observed in children) is significantly compromised [105,106]. We do not have to directly test
in humans if age-compromised MSCs elicit healing effects akin to cells from younger donors. It is possible to deduce an answer based on the responsiveness of Hutchinson-Giffford progeria patients to autologous therapy [106].

The decline in MSC function is typified by an inability to repair injury, and proliferate or differentiate into multiple lineages. If the observed defects [27] are a consequence of deleterious changes in the microenvironment, then the problem might be easily fixable - by exposure to a young unperturbed microenvironment [82]. It can also be fixed by modulating specific pro- and anti-aging factors in the BM micro-milieu. This will involve identification of the specific supportive factors within the BM stem cell microenvironment that are lost or compromised with age and making adjustments. It may also involve transplantation and introduction of niche components into host tissue along with transplanted cells. Conversely, if the defects are solely cell autonomous and intrinsic, development of countermeasures will require a more detailed understanding of underlying mechanisms. This will be followed by pharmacologic and molecular level manipulation of aged ex vivo MSCs in an in vitro system [32,95]. This can be done prior to transplantation for cases where autologous cells are preferred and absolutely necessary. Alternatively, the in vivo BM niche environment can be experimentally targeted to understand how and why aging compromises the efficacy of MSCs. Interactions within the in vivo BM stem cell niche can also be reproduced outside the body to quantify the specific age-associated changes. The ultimate goal will be to manipulate it in the whole organism and halt the age-associated loss of function in MSCs. The report by Asumda and Chase [27] along with a number other recent reports [28,29] demonstrate a definitive association between donor age and defective MSC function. Their specific data set the stage for more mechanistic studies [97,98] detailing a causal role for the aging niche and halt the age-associated loss of function in MSCs. Their specific data set the stage for more mechanistic studies [97,98] detailing a causal role for the aging niche and halt the age-associated loss of function in MSCs. Their specific data set the stage for more mechanistic studies [97,98] detailing a causal role for the aging niche and halt the age-associated loss of function in MSCs. Their specific data set the stage for more mechanistic studies [97,98] detailing a causal role for the aging niche and halt the age-associated loss of function in MSCs.

Acompetence presented here is that the aging niche and consequence deleterious changes that occur in this microenvironment might be the main culprit in MSC aging. The key therefore to overcoming the aging issue in MSC-mediated cell therapy is unlocking and furthering the current understanding of the specific microenvironmental factors that compromise MSCs over time.

**Abbreviations**

BM: Bone marrow; ESC: Embryonic stem cell; MSC: Mesenchymal stem cell; NF-ĸB: Nuclear factor κB; TGF: Transforming growth factor; TLR: Toll-like receptors; VSEL: Very small embryonic like stem cell.

**Competing interests**

The author declares that they have no competing interests.

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