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Review

Characterization of bone marrow-derived mesenchymal stem cells in aging

Natasha Baker [a], Lisa B. Boyette [b], Rocky S. Tuan [a,c,*]

[a] Center for Cellular and Molecular Engineering, Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
[b] Thomas E. Starzl Transplantation Institute, Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
[c] McGowan Institute for Regenerative Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

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ABSTRACT

Adult mesenchymal stem cells are a resource for autologous and allogeneic cell therapies for immune-modulation and regenerative medicine. However, patients most in need of such therapies are often of advanced age. Therefore, the effects of the aged milieu on these cells and their intrinsic aging *in vivo* are important considerations. Furthermore, these cells may require expansion *in vitro* before use as well as for future research. Their aging *in vitro* is thus also an important consideration. Here, we focus on bone marrow mesenchymal stem cells (BMSCs), which are unique compared to other stem cells due to their support of hematopoietic cells in addition to contributing to bone formation. BMSCs may be sensitive to age-related diseases and could perpetuate degenerative diseases in which bone remodeling is a contributory factor. Here, we review (1) the characterization of BMSCs, (2) the characterization of *in vivo*-aged BMSCs, (3) the characterization of *in vitro*-aged BMSCs, and (4) potential approaches to optimize the performance of aged BMSCs. **This article is part of a Special Issue entitled "Stem Cells and Bone"**.

Contents

Introduction

Non-hematopoietic multipotent cells in the stromal compartment of the bone marrow were first identified by Friedenstein and colleagues [1]. These cells have high proliferative potential and the ability to differentiate into chondrocytes, osteoblasts, adipocytes [2], and stromal cells that support hematopoiesis [3]. Furthermore, they have immunomodulatory activity, which may contribute to immune-suppression and tissue healing [4]. Most commonly known as "mesenchymal stem cells" or "multipotent mesenchymal stromal cells" (MSCs), cells labeled as MSCs have been isolated from a variety of extramedullary tissues, are widely believed to serve as a reserve to replace damaged and aged cells, and are well recognized for their potential use in tissue-regenerative cell therapies [5–10]. Importantly, the regenerative function of tissue resident MSCs is said to decline after 30 years of age [6], and their exhaustion is recognized as a potentially important component of aging [11,12]. Our focus here is on the effects of aging on MSCs in the bone marrow, which we refer to hereon as BMSCs.

* Corresponding author at: Center for Cellular and Molecular Engineering, Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, 450 Technology Drive, Room 221, Pittsburgh, PA 15219-3143, USA. Fax: +1 412 624 5544.
E-mail address: rst13@pitt.edu (R.S. Tuan).

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BMSCs may be a particularly important MSC population to consider for three reasons. First, their activity supports that of another distinct population of progenitor cells that give rise to cells of the blood and immune system: hematopoietic progenitors (reviewed in [10]). Second, BMSCs could be used to encourage the repair of extramedullary tissues (reviewed in [13]). Third, BMSCs may influence bone growth and remodeling. They form the osteoblasts that deposit osteoclacin in mineralized bone tissue, and through paracrine stimuli can regulate osteoclasts, which orchestrate osteocalcin release and activation through bone resorption [14,15].

The central integration of bone marrow activity in the body places BMSCs in a position that may be particularly sensitive to aging and age-related diseases. Reciprocally, changes in BMSC activity could contribute to aging in both bone marrow and extramedullary tissues. Therefore, it is vital that we understand more about the biology of BMSCs in the context of aging. Here, we review (1) the characterization of BMSCs, (2) the characterization of *in vivo*-aged BMSCs, (3) the characterization of *in vitro*-aged BMSCs, and (4) potential approaches to optimize the performance of aged BMSCs.

Characterization of BMSCs

The International Society for Cellular Therapy (ISCT) suggested the following criteria for the identification of MSCs: (1) adherence to plastic; (2) differentiation into chondrocytes, osteoblasts, and adipocytes under standard *in vitro* differentiating conditions; and (3) expression of surface markers CD105, CD73, and CD90, in the absence of CD45, CD34, CD14, CD11b, CD79α, CD19, and HLA-DR [16]. However, these criteria, while convenient, have not been completely helpful to the research community because they are based on artificial *in vitro* observations. The immunological markers in particular have not been very helpful, especially for the research of BMSC behavior *in vivo*, as the MSC immunophenotype may be altered artificially by *in vitro* culture (reviewed in [19]). Meanwhile, other potential positive and negative antigenic markers have been suggested. With contrasting combinations of MSC markers described by different researchers, challenges remain in the field regarding BMSC characterization [10,17,18]. The confusion in the literature may be resolved if BMSCs represent a heterogeneous population of progenitors at different stages of differentiation. Indeed, oscillations in immunophenotype characteristics are known to occur during developmental osteogenesis in the chick tibia [5] and BMSCs may reside in three distinct niches within bone, namely, endosteal, stromal and perivascular niches, with different immunophenotypic features within each (reviewed in [19]). Conversely, some researchers have argued that MSCs are a homogeneous population [20].

Those who argue MSCs are a homogeneous population have found CD271 to be the most consistent marker of these cells [20]. However, these authors also admit that levels of CD146 expression are not uniform across the population. Sacchetti and colleagues have shown that at least one subpopulation of CD146+ CD45- cells can be enriched from human BMSC preparations. This CD146+ population is capable of self-renewal and reconstitution of bone and a hematopoiesis-supporting stroma when transplanted into mice [3]. This is important because *in vivo* assay of cellular activity is the most reliable means of BMSC characterization. CD146+ could therefore be one characteristic marker of BMSCs or of a subpopulation of early BMSC progenitor cells. Other researchers have identified CD146 as a marker specific to BMSCs compared to hematopoietic stem cells (HSCs), and shown the *in vitro* chondrogenesis, osteogenesis and adipogenesis of CD146+ BMSCs [21]. Furthermore, an age-related decline in BMSCs expressing CD146 has been reported [22] (For further information on novel MSC markers, see reviews by [10,17,18]).

It has also been suggested that a non-adherent multipotent BMSC/HSC precursor may exist, although this is a more controversial idea. Dominici et al. showed that in mice, a subpopulation of non-plastic adherent bone marrow cells could reconstitute the hematopoietic system in lethally irradiated mice, and also form osteoblasts [23]. They demonstrated that formation of the latter was not a result of cell fusion events by performing cell karyotype analysis. Meanwhile, other investigators have reported isolation of non-plastic adherent bone marrow cells with osteogenic potential [24,25]. In mice, cells with osteogenic potential may be mobilized from the bone marrow to the peripheral circulation to contribute to bone fracture repair in remote locations [23, 26–28]. Cells with osteoblastic potential have also been identified in the circulation of humans [29]. These cells may be relevant to aging and regenerative medicine, as they were reported to increase after bone fracture in humans and are more abundant in the peripheral blood of boys undergoing pubertal growth than in that of men. Others argue that if they exist, these cells are so rare that their relevance in tissue repair is questionable, especially if tissue specific MSCs are present [20].

In summary, there is still not a consensus view on the characterization and lineage markers of BMSCs, and it is possible that BMSCs represent a heterogeneous population with some lineage hierarchy (Fig. 1). If so, it is important to note that the current literature on BMSC aging may encompass age-related changes to subpopulations of BMSCs that are as yet uncharacterized from other populations. With this idea in mind, it is interesting to note that aging causes changes to the clonal composition of the HSC compartment, with a shift towards myeloid bias [30]. Also, immune function has been documented to decline across many different cell types with aging, suggesting that a more inflammatory cytokine milieu may exist in patients of advanced age. This may mean that BMSCs reside in a biologically more challenging environment in people of advanced age, who are most in need of cellular therapies.

BMSCs and aging *in vivo*

Experiments in mice suggest that the aged milieu can suppress the function of adult stem cells [31–36]. Serum isolated from old compared to young mice can suppress the *in vitro* proliferation of stem cells [33]. If expanded beforehand, human embryonic stem cells (hESCs) have at least some capacity to antagonize this effect, suggesting that they produce some factor(s) that can neutralize the anti-proliferative factors in aged serum. Conversely, adult satellite cells appear to lack this ability [33]. Meanwhile, muscle-derived stem progenitor cells (MDSPCs) from aged mice or a mouse progeria model have declined regenerative functions as measured by *in vitro* studies [37]. More importantly, *in vivo*, administration of MDSPCs from young (14- to 21-day-old) mice prevents tissue degeneration and extends the life span and health span of aged mice [37]. These studies suggest that there may be a causal relationship between stem cell aging and organismal aging. Further research is required to confirm and characterize such a relationship, and it remains to be determined if BMSCs are involved in the pathogenesis of organismal aging. It could be hypothesized that BMSCs are susceptible to the hostility of an aged *in vivo* environment, and reciprocally, that BMSC aging may contribute to organismal aging/age-related diseases. For example, it has been speculated that osteoporosis could in part be caused by a deficiency in BMSC osteogenesis [38,39]. This is supported by BMSC atrophy in mouse models of bone aging [15]. However, others have reported no difference between BMSC populations from osteoporosis patients and healthy donors [40].

Disruption of BMSC function may also cause accelerated aging associated with metabolic syndrome, a disorder of energy utilization (see review by [42]). It is speculated that stem cells may become exhausted by demands for adipogenesis in this syndrome, as metabolic syndrome is also observed in lipodystrophies, which are diseases of adipose tissue degeneration [42]. Meanwhile, type 2 diabetes and prediabetes, which are observed in metabolic syndrome, may also cause BMSC dysfunction through the generation of advanced glycation end-products (AGEs). These may accumulate in bone matrix [43] and can suppress proliferation, induce apoptosis, increase intracellular reactive oxygen species (ROS) production, and suppress matrix mineralization