Mesenchymal stem cells, aging and regenerative medicine

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Summary

Tissue maintenance and regeneration is dependent on stem cells and increasing evidence has shown to decline with age. Stem cell based-aging is thought to influence therapeutic efficacy. Mesenchymal stromal cells (MSCs) are involved in tissue regeneration. Here, we discuss the effects of age-related changes on MSC properties considering their possible use in research or regenerative medicine.

Key words: stem cells, mesenchymal stem cells (MSCs), aging, regenerative medicine

Introduction

The interesting overlap between the biology of aging and the biology of stem cells has been reviewed extensively[1-3]. Ageing is accompanied by a progressive decline in stem cell function, resulting in less effective tissue homeostasis and repair. Tissue maintenance and regeneration is dependent on stem cells and therefore, any loss in number or functionality due to aging will likely have a profound effect on our regenerative capacity[4]. Therefore, understanding the basic molecular pathways of age-related stem cell dysfunction in mammals and how stem cell functionality changes with age, including impaired self-renewal and aberrant differentiation potential, have significant implications for regenerative medicine and the goal of extending 'healthspan'[5].
Aging of somatic tissues and organs comes along with a decline of regenerative capacity. Often, tissue homeostasis, regeneration and repair involve the consecutive emergence and parallel integration of new parenchymal cells, which descend from undifferentiated precursors.
In adults, mesenchymal stromal cells contain tissue-specific multipotent stem cells, which can be found throughout the body. Multipotent stromal progenitor cells also known as Mesenchymal Stem Cells (MSCs) are pertinent tissue-specific stem cells in adult beings. The concept of MSC appears to be particularly interesting since this special type of precursor can bring forth a large spectrum of cell types as diverse as bone, cartilage, tendon, or fat precursor cells. MSCs are in the center of attention of many investigators due to easy isolation from many tissues. MSCs capability to differentiate into many cell types makes them a starting point of many new therapies, especially in tissue engineering.
MSCs reside in a complex three-dimensional network, which comprises a plethora of other cell types such as, in the case of bone marrow, hematopoietic stem cells (HSC), adipocytes, and endothelial cells, altogether embedded in distinct extracellular matrix, and within this blend, MSC guide differentiation of hematopoietic precursor cells into mature progeny[6,7]. MSCs appear to exert yet another pertinent function, namely maintaining blood vessel integrity[8,9]. Linked to these presumptions, it can be envisaged that upon tissue damage and injury, MSCs are being activated and/or released from their perivascular niche, in order to support wound healing and tissue regeneration. While extensive research regarding the "Aging-topic" has been undertaken for HSC, and distinct age-related changes and potent molecular mechanisms could be deciphered, distinct details about MSC aging taking place *in vivo* is scarce, simply because we are still lacking consistent knowledge about intrinsic properties in a bodily setting.
However, understanding the process of MSC-aging is crucial for selecting donors for cellular therapies, which is necessary for successful treatment. Cellular changes can be divided into three major groups that include alterations affecting: 1) proliferation rate 2) differentiation capability 3) genome stability. Although many tools have been extensively described to evaluate age-related transformations in MSCs, the aging-process still eludes and further investigations are required. The aim of this review is to take a deep insight into the multidirectional interactions among MSCs, niches and tissues that may contribute to the aged-associated changes. Moreover, significant aspects of MSC-based therapies will be considered to highlight practical limitations that might impair a safe and efficient clinical application.

Aging in MSCs

With advancing age, ascending deficit of cellular proliferation or signal transduction control in MSCs may lead to

differentiation process which affects such as the accumulation of fat deposits in bone and muscles, or impaired healing and fibrosis after severe injury, yet also altered hemopoiesis and autoimmunity.

One major feature of aging is an overall decline in regenerative vigor in many parts of the body. Several functional studies have tackled the question of whether age-associated changes would impinge on MSC properties with respect to their inherent regenerative potential. It is generally accepted that MSC span several hierarchical levels in cell repair and maintenance. MSC are considered a cell source for replenishing worn-out bone, and in case of failure, bone becomes prone to developing osteopenia. Patients with osteoporosis exhibit differences in MSCs, even though subtle[10,11]. MSCs are also subject to modulation by the systemic environment[12,13]. Systemically active factors may not only guide fate decisions according to local differentiation cues but may directly impact on the stem cells' fitness.

Analogously to somatic cells, stem cells experience lifelong exposure to substances such as ROS (Reactive Oxygen Species), biological toxins, harmful chemical agents or physical stressors, which taken together may lead to premature ageing or senescence of individual cells, or provokes accelerated cell death, as well as to cellular transformation risk[14-16]. Indeed, gross evaluations of increased production of ROS[17] deviating SOD (Super Oxide Dismutase) activity[18], whole genome gene expression profiles[12,19], and epigenetic signatures[20] have been reported only recently.

In addition, disrupted inflammatory cues may scramble the delicate balance of regulatory networks necessary to govern tissue specific regeneration and remodeling. It has been shown that levels of pro-inflammatory cytokines are increased in older people[24] and slightly elevated levels of inflammatory stimuli are supporting wound and bone healing by supporting osteoblastogenesis[25]. Deviations of the age-associated osteogenic potential of MSCs isolated from aged donors appears to decline while the respective adipogenic differentiation performance remains unchanged, or worse, is found to be enhanced[21-23]. Contrasted to well adjusted levels, an inflammatory overshoot, be it acutely or chronically, favors adipogenic differentiation. This is in line with observations that bone loss, also included in the advance of osteoporosis in the course of autoimmune disorders of bone, is associated, if not caused by inflammatory disorders[26,27]. Thus, dominant aberrations within the MSC microenvironment may arise from systemic chronic inflammation, which as mentioned above occurs regularly in elderly persons. It may also be mediated through unbalanced inflammatory and anti-inflammatory networks as a consequence to life-long antigenic burden or age-related diseases. These circumstances are often circumscribed by the term "inflamm-aging"[28].

To date no molecular markers are available, which specifically reflect the degree of cellular ageing in MSC population. It has been recently shown that CD295 (leptin receptor) was found to increase as a function of intrinsic aging. Cells, which are double positive for the necrosis marker 7-AAD as well as for the apoptosis marker annexin V are generally considered to be dying cells. Consequently, this particular MSC subpopulation, which is cell-death-prone, can be discriminated by an elevated level of CD295 exposed at the cell surface. Conclusively, enhanced CD295 expression marks apoptotic cells. In the context of proliferating MSC, the appearance of CD295 bright cells emphasizes that the rate of cell death corresponds with the number of cells that fail to self-renew. Interestingly, the death rate steadily rises with increasing cellular age. Taken this example into further consideration, it is certainly more valuable to distinguish phenotypic appearances in MSCs, which are being isolated from differently aged healthy individuals to unveil those mechanisms, which actually take place in a natural situation instead of solely studying *in vitro* MSC senescence[29].

A first plain question regarding age-related variations is whether MSC numbers change during adult life span. Considering the declining proliferative potential with age, variance in long-term culture could be referred to the MSC telomere-attrition at high passages and the relative genotoxic stress eventually may contribute to the limited replicative *in vitro* life-span. However the length of telomeric ends, although being significantly higher in children[30] is maintained at a considerable long length in adult age[12,31]. This suggests that expression of telomerase takes place *in vivo* MSCs at very low constitutive levels, or in a transient fashion thereby maintaining the proper structure of chromosome ends. This clearly shows that changes occurring *in vivo* MSCs are only insufficiently described by patchy examinations of MSCs, which are replicative aged in culture. In this context, it is also worthwhile to assume that stem cells exhibit enhanced cellular repair capacities, or other still unveiled protective measures, which allow them to efficiently restore otherwise irreparable damages.

MSCs in regenerative medicine

The aging-area, that is likely to benefit from advances in the biology of adult stem cells, is the emerging field of regenerative medicine. However, therapeutic applications of MCSs to aged tissue repair in the context of diverse clinical conditions, including immunological disorders as well as degenerative diseases, will require an increased understanding of both stem-cell biology and host aged tissue environment as well as the interaction between the two[32].

Currently, MSC clinical applications required the use of high cellular doses (up to several million cells/ patient body weight) together with efficient expansion protocols to generate a large number of cells based on traditional culture techniques[33]. Cultured MSCs into plastic tissue flasks are limited in terms of cell productivity and at least 2 to 3 cell passages are commonly required to achieve clinically relevant cell numbers in an acceptable period of time. Moreover, a rigorous set up of cell characterization assays to assure a safe and clinically effective MSC product is essential[34]. At this regard is important to note that, the effects of extended *ex-vivo* MSC cells obtained by consecutive cell passaging during long-term cultivation may lead to a senescent state of the cultured cells and ultimately can jeopardize MSC clinical safety and efficacy. Overall, MSC-senescence is a complex, finely organized

process at genomic, transcriptomic, epigenetic and proteomic levels[35]. Although several biological processes have been described to be involved in the long-term culture effects of MSC populations, their precise molecular profile is still unknown and reliable molecular targets of cellular senescence are extremely necessary[36]. Genes or molecules involved in senescence pathways, known to be up regulated by senescence signals are of potential use for these biomarkers[37].

However, there is an increasing knowledge that long-term *ex-vivo* cultivation has to be re-considered in order to avoid alterations in the efficacy and safety of the cellular product. Indeed, most of the reported clinical studies used expanded MSCs up to a maximum of 3 or 4 passages[38]. This was also the case of the MSC infusions that were used in the first clinical studies with *ex-vivo* expanded cells (2-3 passages were used) in Portugal to treat GvHD (Graft-versus-Host Disease) and as adjuvant of hematopoietic cell transplantation[39].

Concordantly, growing clinical applications have been focused on the optimization of *ex-vivo* culture conditions for human MSC expansion by using a low oxygen environment (2%)[40] or a microcarrier-based dynamic culture system[41] operating under xenogeneic-free conditions.

However, nowadays a comprehensive control panel to attest MSC product quality is still to be defined and in order to guarantee the continuous advances of MSC-based therapies[42].

At this regard, important improvement have been made by work of Madeira A. et al.[43] showing that after 7 passages BM-MSCs, the proliferative and clonogenic potential were seriously affected, as well as their proteome profile, namely for proteins in the categories “Structural components and cellular cytoskeleton”, “Folding and stress response proteins”, “Energy metabolism”, “Cell cycle regulation and aging” and “Apoptosis”.

Since the approval of stem cell-based therapies by regulatory agencies (EMA or FDA) relies on a full characterization and safety of the cellular product, this study was a solid contribution to the efforts being made in this field, paving the way to the establishment of a proteomic analysis platform as a quality control panel for cultured MSC.

Conclusion

With the proportion of people over age 60 years growing rapidly in industrial countries, innovative regenerative medicine strategies for the elderly population are such a high priority. Age-related modification of MSC properties should be taken into account whenever they are intended for application in research or cytotherapy. Understanding the complex and dynamic interactions at the molecular, cellular and organ level alongside substantial individual variability requires deep investigation of the entire system involving multiple, interdisciplinary approaches. More rapid and reproducible methods are necessary to isolate, expand and better characterize MSC populations. In addition, further investigations are required to identify specific pathways involved in the activation of endogenous joint-associated stem cells that, in combination with MSCs, are important in the regeneration of a complete and functional tissue. Therefore, innovative approaches on stem cells aging in preclinical models are essential before their application for clinical translation.

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