Changes of the functional capacity of mesenchymal stem cells due to aging or age-associated disease – implications for clinical applications and donor recruitment

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Abstract

In contrast to stem cells of embryonic origin, autologous tissue-specific stem cells are easier to introduce into the clinical practice. In this context however, molecular and cellular changes, which alter tissue-specific stem cell properties with age, are of particular interest, since elderly patients represent the main target group for cell-based therapies. The clinical use of mesenchymal stem cells is an emerging field, especially because this stem cell type appears to be amenable for the treatment of a large number of diseases, such as non-healing bone defects and fractures, inflammatory relieve during arthritis, and the repair of suspensory ligament tears. More than that, mesenchymal stem cells provoke effective immune suppression in the context of graft-versus-host disease.

Here, we present a comprehensive overview of the recent findings with special attention to age-related changes of mesenchymal stem cells properties and the consequential impact involved on tissue regeneration and repair, together with the current perception concerning their therapeutic application potential as well as challenges associated with their clinical use.

(165 words)

"Mesenchymal Stem Cell": a proper term?

This designation is used by many investigators. However up till recently, the definition of what a mesenchymal stem cell is, has been sketchy. Most blood cell types emerge from mesodermal derivatives. Therefore, hematopoietic stem cells are of mesenchymal origin. In contrast however, "mesenchymal" is by all rules assigned to non-hematopoietic cells. With respect to the predication „stem cell", the most stringent condition is the long-term potential to self renew, together with the potency of giving rise to progenitor cells, which in due course differentiate further on to form one or more specialized somatic cell types. In fact, mesenchymal stromal cells from various tissues show, when cultured under specific conditions, a broad differentiation potential. Taken together however, doubts about the appropriateness of the term „mesenchymal *stem* cell" have been raised, in particular because it is scientifically inaccurate and potentially misleading. Instead, the term multipotential mesenchymal stromal cell (MSC) has been proposed [1]. It is generally accepted that MSC can provide the housing for stem cells and for various progenitor cell types such as hematopoietic stem cells together with its respective progeny as well as endothelial progenitor cells. There is reason to believe though that MSC also contain a rare population of naive cell types, which are true mesenchymal stem cells.

Characteristics of uncommitted MSC in culture

In much of the literature, adherent fibroblastoid cells from a variety of tissues are termed mesenchymal stem or progenitor cells. These cell isolates are heterogeneous. To put it bluntly, it is highly unlikely that they comprise of only one single mesenchymal cell type. Due to the fact that there has not been a distinct and universal antigenic definition of a MSC, analogous to $CD34^+$ cells for hematopoietic

stem cells, and as there is no universal assay around, analogous to hematopoietic re-population assays, novel methods for the isolation of MSC with both self-renewal and multipotential differentiation capacity have been, and are still being developed. Arnold Caplan was the first to propose the term „mesenchymal stem cell“ for an adherent fibroblastic cell isolated by Percoll® density centrifugation that expresses antigens reactive with the monoclonal antibodies SH2 (CD105) and SH3 (CD73) [2]. Pittenger and colleagues have reported that CD29, CD44, and CD90 are important determinants [3]. Prockop and co-workers discovered a subpopulation of MSC, termed RS cells, which exhibit enhanced proliferation rates, cannot be clearly distinguished from other adherent MSC solely by expression of specific surface markers [4]. At the same time, Simmons and colleagues described the so-called STRO-1 antibody, which identifies an immature population of mesenchymal cells [5]. Ever since, many other determinants have been discovered [6]. Given these observations, the following minimum specifications to generally define MSC have been proposed only recently by a group of peers in the field: (i) plastic-adherence when maintained in standard culture conditions, (ii) expression of surface molecules CD105, CD73 and CD90, and lack of expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR, and last but not least (iii) differentiation potential towards osteogenic, adipogenic and chondrogenic cell types in vitro [7].

Adult stem cells in an aging organism

Stem cells are often defined as cellular entities that are able to continuously self-renew through replication, while in parallel producing tissue-specific precursor cells. Still, it is not fully understood what regulates the capacity of stem cells to continuously replicate, and which might be the prerequisites to successfully bring

forth various cell types at the same time. Despite a lack of in-depth knowledge about many facts, stem cells hold tremendous promise for the treatment of a variety of diseases. In this respect there is reason to believe that besides embryonic stem cells, also adult stem cells will become highly attractive assets for regenerative medicine; e.g. adult stem cells may be employed in multiple ways to heal or regenerate damaged tissue. More than that, adult stem cells are most attractive, since they can be auto-transplanted, i.e. obtained from and re-inserted into the same patient. Apparently, auto-transplantation of cells eliminates potential donor-host immune rejection and disease transmission issues. Therefore, there is an increasing number of methods being developed and utilized under the guise of "adult stem cell therapy" to treat medical problems. Presently the question is widely discussed, whether adult stem cells are at risk to fail in clinical applications, because they may be prone to cellular aging.

Interestingly, it is still an unresolved issue whether and to what extent adult stem cells can cope with intrinsic and/or extrinsic aging processes in the body. It furthermore is not clear, whether stem cells in complex organisms with multiple, highly specialized organs have life spans that exceed that of the organism. Stem cells potentially face extremely long replicative histories, and in due course, they are therefore subject to damage from several intracellular and extracellular sources. Moreover, it is not only the inevitable process of cellular aging stem cells are afflicted with, but there are also pathological incidences that are associated with aging, such as age-associated diseases and frailty [8]. It is generally believed that over the years, cumulative effects are responsible for the aging of tissues. In line with these

assumptions, work performed by many research teams over the last few years, has demonstrated that MSC face extrinsic and intrinsic aging.

MSC Aging

In the particular context of organismic aging, several laboratories have only recently embarked on the characterization of MSC and thus the field is still in its infancy. As a sound basis it is generally accepted by now that MSC can be isolated from a number of fetal and adult tissues. Human trimester fetal MSC have been isolated from blood, liver, bone marrow [9], amniotic fluid [10], placenta [11], and cord blood [12]; these investigations are still ongoing. MSC from the early organism appear to be more naive when compared to their adult relatives [13, 14]. Regarding MSC isolated form adult tissues, bone marrow-derived MSC are the best-studied [15-17]. Besides MSC, which are juxtaposed to trabecular bone [18, 19], also those from adipose tissues come more and more into fashion [20]. Further sources are periosteum [21], synovium [22], blood vessel [23], tooth pulp of extracted teeth [24, 25] and other connective tissue types, such as dermis and muscle [26]. Besides this, there are reports demonstrating the existence of MSC within peripheral blood. These results are debated though and are not always reproducible [27]. MSC isolated from different tissues and organs actually show a high resemblance of phenotypic characteristics. At this point however, it appears most likely that all these cell variants are MSC that exhibit different propensities in proliferation and differentiation capacities. For example, a recent study demonstrated that human adipose tissue contains the highest number of MSC and umbilical cord blood the lowest. In turn, the latter come along with greatly enhanced proliferation capacity, whereas human bone marrow-derived MSC cease growth earlier [28]. Fetal MSC also appear to be less lineage

committed than MSC from adult human individuals [29]. In consequence, it has been assumed that various vital stem cell properties of MSC, such as their tissue regenerative capacity, is by and large different. Limitations are as follows: MSC only divide a finite number of times; they accumulate genetic and epigenetic changes over time; MSC subtypes exhibit tissue-specific, imprinted differentiation capacity. Having said this, it may further be that these limitations directly correlate with donor age, in particular, that stem cell properties decline with age (Figure 1).

Pool size, differentiation capability in older age

MSC can be selected from low-density mononuclear cell isolates based on their characteristics of tightly adhering to the plastic surface of the culture dish and of forming fibroblastic colonies. The respective number, also called colony-forming unit-fibroblastic(CFU-f), can be reliably estimated. Applying this method, there are presently conflicting results regarding the question whether MSC numbers change during life span. Some laboratories report that total CFU-f decrease with age [30-35], others find no significant decline [36-40]. One particular problem with this approach is that there is no agreement on a single, standardized protocol for MSC isolation as well as how to proceed with further analysis of the primary cell isolates. Another issue is that the primary cell isolates are, though at a varying extent, contaminated by other cells, in particular by hematopoietic cells. Lastly, single clones within the heterogenous cultures exhibit greatly varying differentiation potential, which was demonstrated by in vitro cell cloning of human stromal cultures: only around 30% of the CFU-f are multipotential and can thus be considered true MSC [41]. In addition to the aforementioned observations, Muraglia and colleagues reported that the number of bi- or tri-potential colonies declined with age [42]. In line with this, and in particular

regarding the osteogenic capacity of clonogenic MSC, many reports demonstrated a significant decrease in MSC numbers [43, 44]. Besides bone and bone marrow, potential sources of mesenchymal progenitor cells are muscle [45], vessel-associated pericytes [46], and blood [47]. Whether the quantity or quality of these particular cells varies with age, remains to be determined [48].

Self-renewal capacity and MSC potency with advanced age

MSC are commonly expanded in culture. However, human MSC are not immortal in culture. One possible cellular aging mechanism is replicative or cellular senescence. In 1961, Hayflick and Moorhead unveiled that human skin fibroblasts undergo only a limited number of population doublings in vitro (also termed “Hayflick limit”), and furthermore this number decreases significantly with increasing donor age [49]. Senescent fibroblasts produce elevated levels of molecules that are normally secreted in wound repair and infection, such as inflammatory cytokines, proteases and growth factors [50]. The latter may have detrimental consequences for the surrounding tissue and cells. The presence of senescent cells in vivo, which resemble cells having replicatively aged in culture and that eventually have become senescent, could indeed be observed in aged human skin [51, 52] as well as in the vascular system [53]. Thus, it is plausible that stem cells also reach an equivalent state of senescence in vivo, but evidence for this assumption is still missing, while there is no doubt that replicative senescence of MSC occurs in vitro as demonstrated by a number of laboratories [18, 28, 35, 54-56]. The senescent phenotype of MSC includes the following characteristic features: irreversible arrest of cell division (in contrast to quiescence, where this lock is reversible) and resistance to apoptotic death. Furthermore MSC loose their differentiation potential at a pre-senescent state

[18]. One explanation for replicative senescence emerging in stem cells is the downregulation of telomerase enzyme activity [57]. The net impact is a successive telomere shortening with every cell division during DNA replication. The absence of telomerase activity in MSC and as a result thereof, telomere attrition after extensive replication have been observed by many investigators [16, 58]. Also various types of DNA damage and the expression of particular oncogenes can provoke cellular senescence. Furthermore, it is greatly believed that oxidative stress and other extrinsic influences that result from altered extracellular matrix and disturbed cell-cell and cell-matrix interactions can interfere with self-renewal of the stem cell. In turn this may lead to cellular aging, and eventually result in cellular senescence. Last but not least, it is important to mention that chromatin remodelers, factors, which are well known to be required in the control of the cell cycle, apoptosis and differentiation processes, appear to negatively affect stem cell self-renewal properties. For example, the ATP-dependent remodeling complexes that contain Brg1 have been reported to be involved in the induction of senescence of MSC [59].

As of now, there are no conclusive results available whether MSC in vivo also acquire a state comparable to the senescent phenotype in vitro and whether this is being induced through the above mentioned mechanisms. Most notably in the context of this review is though that the maximum number of MSC population doublings, and the proliferation rate of the initial passage of the primary MSC, respectively, appear to be dependent on the age of the donors [30, 54, 55, 57]. Only recently, we could show that the attenuated proliferation potential of MSC from aged donors greatly relies on the withdrawal of cells by cell death. Conclusively MSC pools, which display slowing growth kinetics, also contain an increasing number of dying

cells, which is indicative for MSC accumulating during advancing age that fail to self-renew [60].

One peculiar feature of MSC is their capacity to escape immune recognition and their ability to suppress the activation of T cells. In vitro, MSC suppress lymphocyte alloreactivity in mixed lymphocyte cultures through a human leucocyte independent mechanism [61-63]. Up till now, the potency of modulating immunological processes has for obvious reasons been confirmed and validated only in a limited number of experimental animal model systems (for a recent review see [64]). In the context of these findings, clinical trials have already been commenced, in which MSC have been employed in the treatment of graft-versus-host disease (GvHD) [65]. Clearance of GvHD with third party haploidentical MSC appears to be possible without side effects [66] and a phase II clinical study on steroid-resistant GvHD has been reported only recently [67]. Further examples, in which MSC have been applied in a clinical setting is to enhance hematopoietic stem cell engraftment in the course of bone marrow transplantation [68]; to correct inherited disorders of bone and cartilage [69]; and/or to ameliorate tissue damage after myocardial infarction [70]. Also, attempts are being undertaken to employ MSC as vehicles for gene therapy, e.g. in osteogenesis imperfecta [71-73]. For a complete list of clinical indications presently being tested, please see: www.ClinicalTrial.gov. However, detailed clinical studies with special regard given to host and/or donor age have, to the best of our knowledge, not been undertaken yet.

MSC and age-associated disease

Bone and bone marrow-derived MSC are definitely a versatile cell source, which can be employed to support tissue repair as well as to enhance regenerative processes (Figure 2). Working along these lines, it is generally believed that MSC have the potential to ameliorate or even cure age-related degenerative diseases. Thus, if implemented sucessfully, this may greatly improve the quality of life for elderly patients. However, certain limitations persist and call for careful quality controls during therapy. Further studies are still needed in order to eliminate risk factors before putting MSC into clinical practice. Actually, the entire field of cell-based therapeutical intervention is confronted with issues related to this topic. These particularly include lacking experience of long-term safety, the absence of standardized assays for quality control and the still incomplete knowledge whether allogeneic MSC when applied in sick or elderly patients would also modulate the immune system efficiently [64, 69, 74, 75].

In this context major concerns are focusing on questions regarding the cell source, and of particular interest is the health status of the donor. As a showcase, Scaffidi and Mistelli could recently show that MSC from patients, who suffer from the premature aging syndrome Hutchinson-Gilford disease show altered differentiation capacities. This observation suggests that impaired or altered stem cell potency may contribute to the reduced potential of tissue homeostasis, and ultimately brings about an aged phenotype in an accelerated fashion [76].

The previous example refers to a disease, which is caused by a single factor, videlicet an aberrant form of the nuclear architectural protein lamin A [77]. In contrast to that, the cause of age-associated pathologies such as osteoarthritis (OA) is not as

well defined. OA is the most common rheumatologic disorder and affects over 70% of people over 65 of age and the etiology is not well understood [78, 79]. It is generally accepted though that besides age, multiple factors such as obesity, history of joint trauma and joint dysplasia are involved. Joint resurfacing with tissue engineered cartilage on the basis of isolated chondrocytes was shown to be greatly beneficial. The availability of chondrocytes however is a major constraint of such a therapy. In this respect, MSC represent an alternative cell source as they exhibit chondrogenic potential. There are however contradicting results: Scharstuhl and colleagues found no significant difference in numbers and differentiation potential when irrespective of age or OA etiology, MSC have been isolated from OA patients [80], whereas Murphy and colleagues observe significant reduction of chondrogenic capacity [81]. The latter is in line with data reported by Muschler and colleagues earlier [32].

In the context of osteoporosis, another major age-associated health complication, in which decreased bone formation is an important pathophysiological mechanism resulting in bone loss, the role of MSC aging is also not clearly defined yet. Stenderup and colleagues showed that the number and the proliferative capacity of mesenchymal progenitor cells are maintained in patients with osteoporosis [37]. In contrast to that, Rodriguez and colleagues demonstrated that stem cell growth, proliferative response and osteogenic differentiation of MSC from osteoporotic postmenopausal women are significantly affected [82]. Since MSC are already employed to augment large bone defects by grafting engineered osseous tissue derived from explanted osteoprogenitors, specific means, which clearly define those cellular properties may greatly enhance the outcome of these modern therapeutic strategies.

These few examples clearly show that it is presently not clear at all whether MSC, which have been isolated from elderly (yet healthy) individuals can be safely implemented in clinical practice, primarily because aged organisms are faced, and this to a greatly varying extent, with the life-long accumulation of potentially deleterious impacts. Therefore, transplantation of naive stem cells, which are capable of producing progeny, and which will actually continue to do so in situ post implantation, without careful examination of putative alterations of their cellular characteristics prior to use, seems highly risky. Similar to other cell types, it is indispensable to perform extensive quality controls for all MSC preparations to be employed in therapeutic applications. This has to include phenotypic, functional and genetic characterization. For the latter, many academic and clinical researchers are currently characterizing MSC by means of modern, state-of the art technologies. It can be anticipated that these unbiased data analyses, using genomic and proteomic methods, will elucidate distinct molecular parameters, which prognosticate the performance of individual MSC preparations, be it primary isolates or cells expanded in vitro.

Concluding remarks

The prospective clinical use of MSC holds enormous promise for the treatment of a large number of degenerative and age-related diseases. However, the challenges and risks for cell-based therapies are multifaceted. The theoretical health risk for patients receiving autologous MSC can be hardly anticipated and the proper ways of manipulating the cells ex vivo are currently a matter of intensive investigation.

Careful pre-administration safety monitoring as well as close monitoring of the patients is essential for this novel form of therapy. Regulatory bodies such as the US Food and Drug administration [83] and the European Union (http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/l_324/l_32420071210en01210137.pdf) have recently established a set of regulations for cell-based therapeutics. The International Society for Stem Cell Research (ISSCR) has set up a "task force on clinical translation of stem cells" (http://www.isscr.org/committees/committeeResults.cfm?CommitteeName=COMMITTEE/CLINTRANS) since more distinct directives are needed to progress effectively in translating this new technology. Only with continuous and open interactions between investigators, who provide appropriate analytical methodology and detailed understanding of the dynamic nature of stem cells, research institutions that capitalize and endow peer-to-peer network environment and sustain complex logistics for clinical studies, together with regulatory bodies, efficient and safe cell-based therapies will become routine, in particular for patients suffering from age-associated diseases or frailty.

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Figure Legends

Figure 1: Emergence of MSC diversity during life span. Early in life, most, if not all uncommitted MSC exhibit multipotential differentiation capacity, and their developmental fate is tightly controlled. In due course, additional types of mesenchymal precursors emerge as stable subpopulations. The rate of cell death is gradually increasing (MSC^{dying}). Other MSC accumulate various forms of damage, which cannot be compensated by cellular repair (MSC^{degen}). Due to an age-dependent change in the activity of bioactive differentiation cues, be it their respective availability, or alterations in cellular response, or because of chronic, systemic inflammation, a subpopulation of uncommitted MSC becomes manifest, predetermined mesenchymal precursors (MPC). In total, only little decline of mesenchymal progenitor cell numbers is envisaged at advanced age. However, the number of truly naive, multipotential MSC steadily declines.

Figure 2: Model of extrinsic feedback control on mesenchymal tissue regeneration by MSC progeny. MSC reside in their niches within mesenchymal tissues or organs. MSC are involved in regeneration and repair by bringing forth progenitor cells. In addition, MSC nurture stromal compartments with cells, which themselves are important regulators for stem cell renewal, in particular in the case of hematopoietic stem and precursor cells. Lastly, MSC exert immune modulatory properties. These processes are intricately dependent on the supervision of the niche. These constantly undergo changes not only because of cellular metabolic activity, but also due to pathologic insults, biological aging or tissue damage.

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Figure 1

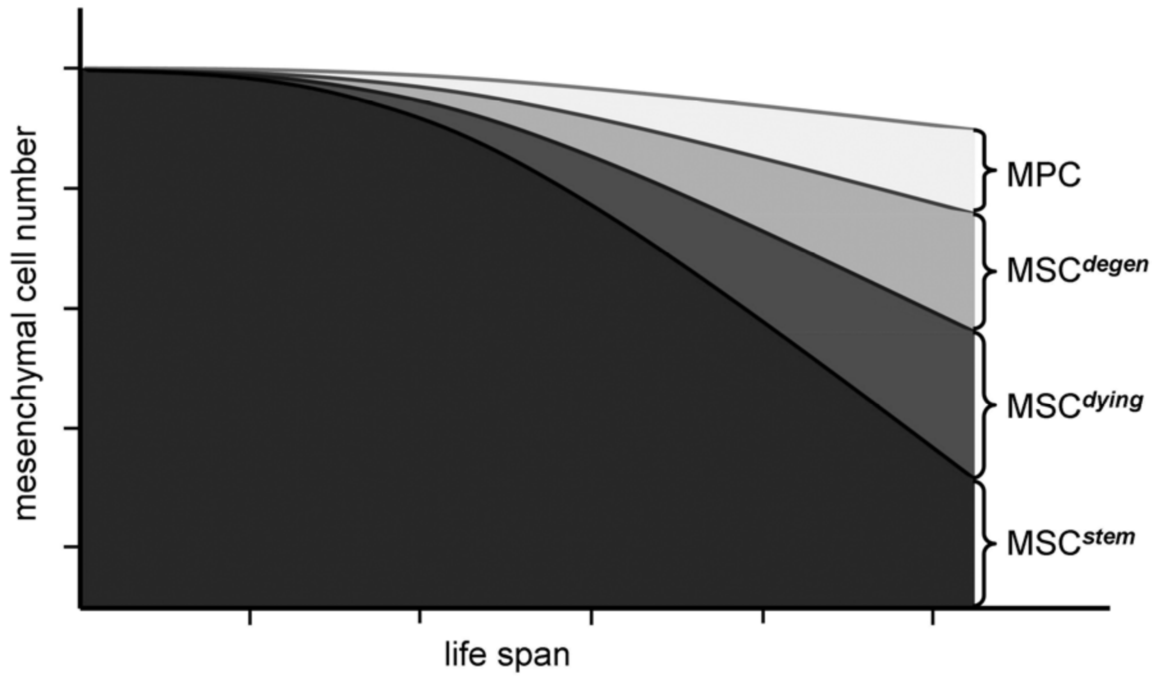

Figure 2