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Review Article

MSCs' Rejuvenation Strategies with an Immediate Translational Potential

Ilaria Roato, Federico Mussano*

Department of Surgical Sciences, University of Turin, Turin, Italy

ABSTRACT

Mesenchymal Stem/Stromal Cells (MSCs) are multipotent cells indispensable for the homeostatic maintenance and regeneration of a large variety of tissues, almost ubiquitous in human body. Despite the large number of clinical trials proving consistently MSCs' safety, only a few protocols have received regulatory approval. As main causes of such a reduced efficacy, cells heterogeneity limiting the standardization of the therapies and the onset of senescence have been invoked. To address this shortcoming, researchers have been working on several strategies capable of delaying or even hopefully reversing the senescent phenotypes of MSCs. In the present short review, the authors focused only on the reduction of ROS and the administration of anti-senescent drugs on the grounds of their rapid translation potential. Indeed, off target effects of anti-senescent therapies may even include oncogenesis and thus require the uttermost attention to safety issues besides efficacy evidence. More studies are needed to elucidate the physiologic mechanisms underpinning stem cell aging, aiming at developing novel methods that may extend the functional lifespan of stem cells in a safe, reproducible, effective and tuneable way.

Keywords: Stemness; Cell cycle; Senescent; Reactive oxygen species; Regenerative potential

INTRODUCTION

Mesenchymal stem cells, also known as Mesenchymal Stromal Cells (MSCs) are multipotent non-hematopoietic stem cells that represent a reservoir indispensable for the homeostatic maintenance and regeneration of a large variety of tissues throughout the individual lifespan, owing to their capacity of keeping their "stemness", while differentiating into the required histotype [1]. Ubiquitous in the human body [2], MSCs have been defined through "minimal criteria" by Dominici et al. in 2006, as follows: a) Tissue culture plastic adherent; b) Positive (≥ 95%) for surface antigen markers CD105, CD90 and CD73 while also negative (≤ 2%) for CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR; and c) Capable of differentiation to adipocytes, chondroblasts and osteoblasts [3].

Oral cavity harbours a variety of MSCs [4], but they are prevalently found in bone marrow, umbilical cord and adipose tissue. Indeed, these three sources of MSCs have been utilized in 77% of the registered clinical trials, according to Zhouh et al. [5]. Nonetheless, only a few MSC-based protocols have received regulatory approval [6]. Among the possible causes of this shortcoming, there are cells' heterogeneity limiting the standardization of the therapies and the onset of senescence [7] that is the object of the present mini-review.

LITERATURE REVIEW

Cellular senescence

Cellular senescence regards properly mitotic cells and is defined usually as a "permanent cell cycle arrest in metabolically active cells" [8], the full range of consequences thereof are yet to be exhaustively elucidated. Senescence is important to control cell growth, as it protects against tumorigenesis by preventing that cells with stress-induced damage enter the replicative cycle [9]. As a physiological homeostatic mechanism senescence is implicated in tissue repair [10], while its impairment leads to aging-related inflammatory conditions such as osteoarthritis and Alzheimer's disease [11,12].

Recent studies report that senescent MSCs show reduced regenerative potential, impaired migration and altered paracrine secretion [13]. Transplanted senescent cells not only exhibit less

Correspondence to: Federico Mussano, Department of Surgical Sciences, University of Turin, Turin, Italy; E-mail: federico.mussano@unito.it

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efficient therapeutic function but may even result detrimental [14], posing a major issue for MSC treatments. Thus preventing or at least reducing, stem cell senescence is emerging as a paramount task to improve the efficacy of future clinical applications.

The cell cycle arrest of senescent MSCs is mainly dependent on replicative and oxidative stresses.

Due to the shortening of telomeres up to a critical length of 10 kilo base pairs, replicative senescence affects MSCs that, lacking detectable telomerase activity, are estimated to undergo a maximum of 30 population doublings prior to reaching the critical telomere shortening [15]. Indeed, senescent MSCs increase their proportion with each replicative passage and they reach often senescence prior to their expected replicative limit, as a result of other stress factors that cause premature senescence, such as DNA-damaging Reactive Oxygen Species (ROS) [8].

Natural defense mechanisms against ROS, which are physiological products of the aerobic metabolism, may not be sufficient to avoid senescence owing to oxidative stress. Senescent MSCs produce ROS with a positive feedback loop in which the more ROS the worse the senescence and the consequence molecular damage. Since a remarkable cell expansion is mandatory to treat a person, who needs one to two million cells per kilogram of body mass and the yield of MSCs in their niche is estimated as low as one in every 50 million cells, it's relevant to delay as much as possible senescence.

MSCs' senescence features

Senescing cells exhibit Senescence Associated Secretory Phenotype (SASP), *i.e.*, a multitude of se-cretory proteins, including inflammatory and matrix-modelling signaling molecules, released with autocrine and paracrine effects. Among the major SASP factors it is convenient to enumerate interferon-β, Vascular Endothelial Growth Factors (VEGFs), TGF-β family ligands, C-C motif ligand 2 and 20, along with radiation induced senescence pro-inflammatory interleukin-6 and interkeukin-8. Senescent MSCs can be distinguished from healthy cells, also based on immunogenicity, that allows their removal *in vivo*, but obviously not *in vitro*, hence the importance of timely detection and removal of senescent cells to ensure MSCs' potency in cell culture.

Positive staining for SA-β-gal activity is universally known as a feature of senescent cells, along with the persistent expression of p53, p21 and p16 proteins. Likewise, a cell morphology enlarged and flattened, due to the decoupling of cell division and cell growth, is typical of cell senescence. Although no universal marker for senescence has been identified, the concomitant decrease of the adhesion molecule CD146 and higher expression of decoy TRAIL receptor CD264 have been associated with senescent MSCs. Recently, also autofluorescence has been reported as a feature of senescence. Finally senescent MSCs display nuclear changes such as the loss of lamin B1 that even precedes SASP and SA-β-gal activity and the onset of Senescence-Associated Heterochromatic Foci (SAHF). These punctate nuclear structures are probably condensed chromosomes that depend on p53 and Rb activation.

MSCs' rejuvenation strategies

The therapeutic potency of MSCs is hindered by the functional alterations induced by senescence, including decreased migration, proliferative and differentiative potential. Luckily, at least some of these features seem reversible. A series of approaches are described in the following section focusing on the techniques that are likely to achieve clinical translation.

Reduction of ROS

Since hypoxic culture conditions mimicking the biological niche of MSCs decrease senescent phenotype, hypoxic pre-conditioning was proposed to rejuvenate MSCs before transplantation. Antioxidants scavenge local ROS delaying the onset of senescence, indeed when aged MSCs are treated with reduced glutathione and melatonin, they reacquire early-passage stemness and migratory capabilities, while lowering the expression of p16, p21 and p53. Ascorbic acid, which is a prototypical antioxidant and also an mTOR signaling inhibitor, has been proven capable of extending greatly the MSC expansion limit. Caution is suggested, on the other hand, as its prolonged usage may affect differentiation capacity. Indeed, at low intrinsic levels ROS are beneficial for MSCs' osteogenesis and their excessive removal can result in a paradoxical induction of senescence in proliferating cells, as well as in suppression of adipogenic potential.

Anti-aging molecules/drugs

Among the most promising anti-senescent drugs, rapamycin is a potent inhibitor of the mechanistic Target of Rapamycin Complex One (mTORC1) protein kinase, that showed anti-aging properties such as restoring differentiation and proliferation potential, rescuing nuclear membrane deformation, reverting morphological changes, SA-β-gal activation and p53/p21 expression in diverse model systems, including stem cells derived from a progeroid mouse model. Note that these beneficial effects rely on the inhibition of mTORC1, while most of the unwanted side effects are due to the inhibition of mTORC2, which has paved the way to exploring a therapeutic strategy targeting selectively mTORC1 [16]. A number of clinical trials are testing the safety and efficacy of rapamycin in attenuating the ageing process, but up to now no human data have supported its use as geroprotector, which is a noteworthy translational knowledge gap.

Metformin, on the contrary, is being trialed in humans as the first geroprotective drug and in adipose derived MSCs, it reduced replicative senescence likely owing to its ROS scavenging activity, which is consistent with the reported activation of AMP-activated protein kinase implicated in mitochondrial homeostasis. Indeed, cell rejuvenation is possibly attained by restoring mitochondrial function.

As regards the drugs targeting mitochondria having senotherapeutic potential, besides the antioxi-dant mitoquinone, it is worth remembering melatonin. When compared to untreated

controls, melatonin-treated senescent MSCs seem to possess enhanced therapeutic potential, sustaining better ischemic recovery and neovascularisation in murine models. From a molecular point of view, melatonin increases Sirtuin 1 (SIRT1) expression, while inhibiting at the same time ROS accumulation and it also activates mitophagy which can clear damaged mitochondria [17,18]. Likewise, other molecules promoting SIRT1 activity, such as resveratrol or Nicotinamide Adenine Dinucleotide (NAD), ameliorate the "stemness" properties of early-passage MSCs, while rescuing the functional impairment of senescent cells [19]. Unfortunately, this outcome is achieved only at high drug concentrations or with prolonged use. To counteract this pitfall, Wang et al. have recently proposed a "targeting nanoplatform with a strong affinity for senescent MSCs through conjugation with anti-Kremen1", that may be useful in future nano-therapeutics.

DISCUSSION

Finally, based on the observation that DNA methylation is highly associated with ageing-related diseases, DNA Methyltransferase (DNMTs) inhibitors are a category of anti-senescence drugs that have been acquiring interest in the last few years. A very promising candidate belonging to DNMTs inhibitors, RG108 was first tested in human Bone Marrow Mesenchymal Stromal Cells (hBM-MSCs), with encouraging results. These data were confirmed in Periodontal Ligament-Derived Stem Cells (PDLSCs) harvested from patients with periodontitis, suggesting the use of RG108 and possibly other anti-senescent molecules, in regenerative protocols. Although MSCs are widely regarded as a powerful and safe therapeutic option, their actual translation to clinics has been limited up to now. This is due to a series of causes among which cellular senescence, a natural response to accumulated stress-induced damage, represents a key hurdle in MSC usage. To address this shortcoming, researchers have been working on several strategies capable of delaying or even hopefully reversing the senescent phenotypes of MSCs [20].

CONCLUSION

In the present short review, the authors focused only on the reduction of ROS and the administration of anti-senescent drugs on the grounds of their rapid translation potential. Indeed, off target effects of anti-senescent therapies may even include oncogenesis and thus require the uttermost attention to safety issues besides efficacy evidence. More studies are needed to elucidate the physiologic mechanisms underpinning stem cell aging, aiming at developing novel methods that may extend the functional lifespan of stem cells in a safe, reproducible, effective and tunable way.

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