Mesenchymal stem cells-based therapy as a potential treatment in neurodegenerative disorders: is the escape from senescence an answer?

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Abstract
Aging is the most prominent risk factor contributing to the development of neurodegenerative disorders. In the United States, over 35 million of elderly people suffer from age-related diseases. Aging impairs the self-repair ability of neuronal cells, which undergo progressive deterioration. Once initiated, this process hampers the already limited regenerative power of the central nervous system, making the search for new therapeutic strategies particularly difficult in elderly affected patients. So far, mesenchymal stem cells have proven to be a viable option to ameliorate certain aspects of neurodegeneration, as they possess high proliferative rate and differentiate into multiple lineages. However, accumulating data have demonstrated that during long-term culture, mesenchymal stem cells undergo spontaneous transformation. Transformed mesenchymal stem cells show typical features of senescence, including the progressive shortening of telomers, which results in cell loss and, as a consequence, hampered regenerative potential. These evidences, in line with those observed in mesenchymal stem cells isolated from old donors, suggest that senescence may represent a limit to mesenchymal stem cells exploitation in therapy, prompting scholars to either find alternative sources of pluripotent cells or to arrest the age-related transformation. In this present review, we summarize findings from recent literature, and critically discuss some of the major hurdles encountered in the search of appropriate sources of mesenchymal stem cells, as well as benefits arising from their use in neurodegenerative diseases. Finally, we provide some insights that may aid in the development of strategies to arrest or, at least, delay the aging of mesenchymal stem cells to improve their therapeutic potential.

Key Words: aging; neurodegenerative disorders; telomere shortening; MSCs; cellular therapy

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Introduction
Aging is the most prominent risk factor for the occurrence of neurodegenerative diseases among others, including oxidative stress (Keller et al., 2005; Jain et al., 2011), telomere length (Harris et al., 2006), genetic mutations (Anderton et al., 2002) and head injury (Maiese et al., 2008). In the United States there are over 35 million of people with a mean age of 65 years and even older, that mainly die from age-related diseases (Drago et al., 2011). Aging increases susceptibility of people to environmental stressors, thereby increasing the chance to develop neurodegenerative conditions, most likely because the self-repair ability is compromised and tissues and/or organs undergo a progressive decline (Musumeci et al., 2014a). The aging process is associated with a number of structural, biochemical, functional and neurocognitive changes in the brain. The structural changes include expansion of cerebral ventricles, regional decreases in cerebral volume (Raz et al., 2005), loss of neural circuits and reduced brain plasticity (Burke and Barnes, 2006; Kolb and Gibb, 2011), thinning of the cortex (Shahani et al., 2006), decrease in both of the grey and the white matter volume (Bartzokis, 2011), changes in neuronal morphology (Sowell et al., 2003) and formation of neurofibrillary tangles (Hedden and Gabrieli, 2004; Neill, 2012). Among the age-related biochemical changes there are marked alterations in neurotransmitters and their receptors. Significant decreases in dopamine receptors D1, D2, and D3 (Wang et al., 1998; Kaasinen et al., 2000) and decreasing levels of different serotonin receptors and their transporters such as 5-hydroxytryptamine transporters (5-HTTs) (Chang and Martin, 2009; Chang et al., 2009) have been repeatedly reported. Among the neuropsychological changes, alterations in orientation (Benton et al., 1981) and memory (Hof and Morrison, 2004) are the most common ones. Moreover, many age-related neurodegenerative diseases are characterized by...
accumulation of disease-specific misfolded proteins in the central nervous system (CNS) (van Ham et al., 2009). These include β-amyloid peptides and tau/phosphorylated tau proteins in Alzheimer's disease (AD), α-synuclein in Parkinson's disease (PD), superoxide dismutase (SOD) in amyotrophic lateral sclerosis (ALS) (Durham et al., 1997), and mutant huntingtin in Huntington's disease (HD) (Scherzinger et al., 1997). The association between age and protein misfolding is not clear yet, but it is probably related to alterations of molecular mechanisms triggered by aging cells, such as telomere shortening, cells shrinkage and decline of quality control over protein synthesis mechanisms (Hung et al., 2010; Than- an et al., 2014) (summarized in Figure 1).

Telomeres are an evolutionarily conserved repetitive nucleotide sequences (TTAGGG) localized at the end of each chromosome, that are folded into a T loop structure by a protein complex called shelterin (Stewart et al., 2012). Telomeres play four fundamental roles: protecting genetic information from erosion during DNA replication; protecting DNA from damage; serving as a binding site for DNA repair proteins; and providing information about the cell proliferation history (Stewart et al., 2012; Musumeci et al., 2015; Giunta et al., 2015). During each round of DNA replication, telomeres of cultured cells typically lose about 50–200 bp (Allsopp et al., 1992). The telomere length is the sand glass of the cell since it specifies the number of divisions a cell can undergo before it finally dies; thus, it indicates the cell proliferative potential. Telomere shortening leads to the attainment of the so-called 'Hayflick limit', which indicates the transition of cells to the state of senescence. Following this step, cells progressively enter a state of crisis, which is accompanied metabolic disturbances that culminate in massive cell death. An enzyme able to maintain telomere length is called telomerase. Telomerase plays a pivotal role in the pathology of aging and cancer by maintaining genome integrity, controlling cell proliferation, and regulating tissue homeostasis. Telomerase is essentially composed of an RNA component, the telomerase RNA or TERC, which serves as a template for telomeric DNA synthesis, and a catalytic subunit, telomerase reverse transcriptase (TERT). The canonical function of TERT is the synthesis of telomeric DNA repeats, and the maintenance of telomere length. However, accumulating evidence indicates that TERT may also exert some fundamental functions that are independent of its enzymatic activity (Verdun and Karlseder, 2007) (please refer to Figure 2). A reduction in telomerase expression contributes to telomere shortening in mitotic cells, while high levels of the enzyme in mesenchymal stem cells (MSCs) contribute to their 'immortal' phenotype (Rubtsova et al., 2012). The phenomenon of telomere shortening is closely associated with aging itself, but it has been widely demonstrated that cells can also undergo premature aging due to several factors such as oxidative stress, inflammation and infections, which are able to speed up this process and determine age-related dysfunctions (Hung et al., 2010; Jenny, 2012; Kong et al., 2013; Kota et al., 2015). Therefore, given the involvement of these factors (and in particular of oxidative stress) in the development of neurodegenerative/age-associated diseases, it becomes of primary importance to also gain more insights on the underlying mechanisms triggered by these stressors, as this could serve to improve current therapeutic strategies based on the use of MSCs to treat neurodegenerative conditions.

A reason why telomeres are the preferred targets of oxidative insult seems to be primarily related to their DNA composition, which tends to be rich in guanine residues (Coluzzi et al., 2014). Indeed, the high incidence of guanine bases promotes the generation of alterations to DNA bases to species called 8-oxoguanine (8-oxoG), which, if not repaired, may lead to single or double strand breaks, mutations or even genomic instability (Grollman et al., 1993). Of interest, genomic instability, oxidative stress and ageing are not to be considered as independent causative factors in telomere shortening, but need to be considered as interconnected phenomena. Consistent with this theory, convergent data has identified an accelerated Wnt/β-catenin cascade activation as a common denominator triggered by these insults. Activation of this pathway reduces MSCs proliferation potential, hampers telomerase activity and drives a cellular shift of MSCs towards a differentiated/senescent phenotype (as elegantly reviewed by Fukada et al., 2014). In the light of these evidences, it is auspicious that strategies aimed at dampening the occurrence of these detrimental events in neurons or to block the Wnt/β-catenin intracellular pathways could have the potential to significantly impact the senescent process, including premature telomere shortening.

Leukocyte Telomere Length (LTL), a Biomarker in Neurodegenerative Disorders

Early neuronal cell death is a feature of neurodegenerative disorders and reduced telomere length has been associated with premature cellular senescence. Studies have shown that reduced telomere length in peripheral blood is associated with the incidence of illnesses associated to the aging phenotypes, such as dementia (Thomas et al., 2008), neurodegenerative diseases such as HD and genetic neurovascular diseases such as ataxia telangiectasia (AT) (Metcalfe et al., 1996; Kota et al., 2015)). Since LTL is reflective of global cellular morbidity and mortality, it has been proven that it could be used as a useful tool to screen neurodegenerative disorders (Sahin and DePinho, 2012). It is worth emphasizing, however, that leukocytes include diverse cell populations that play complementary roles in tissue homeostasis and responses to infections and diseases, and the possibility exists that simply monitoring LTL may lead to misleading results. Indeed, the three major classifications of leukocytes are granulocytes, lymphocytes, and monocytes. These populations have different telomere lengths and erosion rates as a result of differences in telomerase activity, proliferation history, and telomere trimming (Stewart et al., 2012). These differences have to be carefully taken into account when considering a possible study of age-related processes in neurodegenerative disorders. However, a recent study has consistently showed that the LTL was reduced in individuals suffering from neurodegenerative disorders as described above, suggesting
that the phenomenon of telomere shortening could at least be partly implicated or could contribute to the triggering of pathological pathways activated in these diseases (Kota et al., 2015). In dementia, the reduced telomere length has been attributed to oxidative stress, aberrations in mitochondrial homeostasis, deficient DNA repair mechanisms, and decreased DNA methylation status (von Zglinicki, 2002; Blasco, 2007; Gallkowski et al., 2008; Coppè and Migliore, 2009; van Groen, 2010; Sahin and DePinho, 2012). Nevertheless, the precise mechanism for this attrition needs to be studied further. Interestingly, a review of data from literature suggesting the potential use of LTL as a biomarker in AD and PD showed to be inconsistent in both cases, since the number of studies reporting no association between LTL and disease states almost overlapped the ones indicating a correlation between LTL shortening and neurodegeneration (Eitan et al., 2014). Interestingly enough, a recent study reported an even longer LTL in PD patients, associating short telomeres with reduced risk of PD (Schürks et al., 2014). The reason of these inconsistencies could be dependent on the population number used in these studies, as the low number of patients together with the inter-individual variability may often result in significantly reduced statistical power, and purportedly unreliable results. It has been shown that variability in LTL in individuals can be induced by different factors such as chronic stress, diet, lifestyle, chronic inflammatory status and hormone levels (Liu et al., 2010; Broer et al., 2013). The interaction between these factors and genotype can also play a role in LTL variability (Takata et al., 2012). Certainly, further investigation in this field are needed to clarify the precise role and diagnostic or therapeutic potential of LTL in neurodegeneration.

MSCs-based Therapy for Neurodegenerative Disorders

The limited regeneration power of the CNS represents a major challenge for the development of new therapeutic strategies efficacious to promote its functional repair. MSCs have been proposed as a viable therapeutic tool for degenerative disorders as they possess high proliferative ability and they are able to differentiate into multiple lineages (Mobasher et al., 2014; Musumeci et al., 2014; Tanna and Sachan, 2014). MSCs can differentiate into neuron-like cells and determine a paracrine effect by modulating the plasticity of damaged host tissues; by secreting neurotrophic and survival-promoting growth factors that inhibit apoptosis and promote neurogenesis, glial scar formation, immunomodulation, angiogenesis and neuronal and glial cell survival; by restoring synaptic transmitter release; by integrating into existing neural and synaptic networks; and by re-establishing functional afferent and efferent connections (Siniscalco et al., 2010; Teixeira et al., 2013; Ooi et al., 2014). In addition, low immunostimulating and high immunosuppressive properties make MSCs a suitable source for cellular therapy (Abumaree et al., 2012; Kwon et al., 2014). Another point in favor to MSCs employment in therapy is that cells can be transplanted directly without any prior genetic modification or reprogramming, and are able to migrate to the tissue injury sites (Amado et al., 2005). MSCs have also been proven to be useful for the treatment of pathologies in which tissue damage is caused by oxidative stress and thus in those pathologies linked to stress-induced telomere shortening and premature aging, where MSCs are likely to be more resistant to oxidative insult than normal somatic cells (Benameur et al., 2015). This feature is particularly important since it makes MSCs an interesting and testable model for the treatment of age-related neurodegenerative disorders.

Currently, there is a great interest towards the use of MSCs in pioneering therapies aimed at treating chronic and progressive neurodegenerative diseases, which are currently incurable and whose attempts to find disease-modifying therapies have failed, such as AD, PD, ALS and HD. It has been shown that after transplantation into the brain, MSCs promote neuronal growth, decrease apoptosis, reduce the levels of free radicals, stimulate the formation of new synaptic networks from damaged neurons by supporting axonal outgrowth, modulate neuroinflammatory activities and promote proteosomal degradation of ubiquitinated misfolded proteins (Caplan and Dennis, 2006; Mezey, 2007; Uccelli et al., 2011). Through paracrine mechanisms, MSCs are also able to interact with neighbouring damaged host cells and influence their microenvironment, by sharing proteins, RNAs and even mitochondria (Spees et al., 2006; Olson et al., 2012). As a proof-of-concept, Mazzini et al. demonstrated that MSCs can decrease motor neuron cell death through paracrine actions when implanted into the CNS of ALS patients (Mazzini et al., 2003; Boucherie et al., 2009). Recently, the paracrine properties of bone marrow-derived MSCs (BM-MSCs) have been also shown in rat model of AD, suggesting their potential therapeutic role in this disease (Salem et al., 2014). The potential efficacy of human MSCs (hMSCs) has been also confirmed recently, as treatment succeeded to ameliorate some behavioral defects observed in a rodent model of HD, hence demonstrating that xenologous transplantation of hMSCs could be considered a potentially successful approach to counteract neurodegeneration caused by HD, and perhaps other CNS disorders (Hosseini et al., 2014).

MSCs can be readily isolated from various tissues, show high plasticity and are capable to differentiate into many functional cell types (Woodbury et al., 2000; Krampera et al., 2007; Singec et al., 2007). Numerous studies have shown that BM-MSCs can differentiate into cells that display neuronal or even dopaminergic characteristics both in vitro and in vivo (Ni et al., 2010; Zeng et al., 2011). A recent study reported that mouse BM-MSCs provided neuroprotection by secreting a key factor, prosaposin, a molecule capable of rescuing mature neurons from apoptotic death. The secretome of BM-MSCs showed to reduce toxin-induced cell death in cultures of rat pheochromocytoma cells, human ReNcell cortical neurons, and rat cortical primary neurons (Li et al., 2010). Unfortunately, the medical procedure to obtain BM-MSCs from the bone marrow is invasive and definitely painful to patients. Therefore, efforts have been made
to find more practical alternatives. Indeed, recently other MSCs sources have gained clinical interest for use in regenerative medicine; and adipose tissue represents one of these sources with a broad spectrum of benefits. Human adipose tissue represents a readily available autologous source of MSCs (Ghasemi and Razavi, 2014). Human adipose tissue-derived MSCs (hAT-MSCs) retain morphological, phenotypic and functional characteristics resembling those of BM-MSCs (Zuk et al., 2002), are stable over long term culture, expand efficiently in vitro and possess multi-lineage differentiation potential (Zuk et al., 2001; Musumeci et al., 2011; Choudhery et al., 2013; Musumeci et al., 2014b). Latest observations suggest that transplantation of hAT-MSCs into the brains of elderly mice improved both locomotor activity and cognitive functions. Transplanted cells rapidly differentiated into neurons and in part, into astrocytes, and produced choline acetyltransferase proteins, restoring acetylcholine levels in the brain. Moreover, transplantation of hAT-MSCs restored neuronal integrity by stimulating the release of neurotrophic factors by neighbouring cells (Park et al., 2013). In this regard, an aspect to be considered in MSCs therapies is that it is now well-recognized that many pleiotropic molecules endowed with neuroprotective potential, including some neuropeptides produced locally by resident glial cells or neurons (i.e., pituitary adenyl cyclase activating polypeptide and/or vasoactive intestinal peptide), when stimulated by neighbouring cells (i.e., implanted MSCs) may prevent cognitive decline caused by aging (Pirger et al., 2014), facilitate nerve recovery after injury both in the CNS (reviewed by Waschek, 2013) and the periphery (Tamas et al., 2012), stimulate remielination processes and glial regenerative support to neurons (Castorina et al., 2014, 2015) and are even capable to prevent retinal damage and maintain retinal barrier properties (Giumaa et al., 2012; Scuderi et al., 2013) or impede oxidative insults (Castorina et al., 2012), a broad spectrum of physiopathological events that, at different degrees, are negatively impacted by senescence. Even more interesting, combinatorial administration of these molecules with MSCs has been suggested to support spinal cord recovery after damage (Fang et al., 2010), inferring on the mutual reciprocity between the two, especially desirable to complement the existing gaps determined by the single therapeutic employment of MSCs in aged patients affected by neurodegenerative disorders.

Another source of MSCs that has captured minor scientific interest is represented by dental pulp stem cells (DPPSCs). DPPSCs have also been recognized as capable to differentiate into a variety of cell lineages (Zhang et al., 2006; Huang et al., 2009), but more studies are required to better define their potential. Other sources of stem cells are that obtained from human-exfoliated deciduous teeth (SHED), which have been shown to contain multipotent stem cells (Miura et al., 2003) The importance of SHED is that they are derived from a tissue similar to the umbilical cord. Notably, both kinds of DPPSCs can be induced to differentiate into neuron-like cells and be transplanted in brain injury and/or neurodegenerative disease animal models to conduct neuroregeneration studies (Sakai et al., 2012; Tamaki et al., 2012; Yamagata et al., 2013). Based on these findings, it is plausible to believe that the extracted teeth, considered a common waste product from dental extraction procedures, could be employed in the future to exploit in tissue engineering strategies as a promising substitute of BM-MSCs.

Limits of MSCs-based Therapy
A major issue that has significantly limited the use of MSCs-based therapy is the low yielding of viable MSCs from donor tissue. In fact, in order to harvest sufficient MSCs to procure some clinical benefits cells need to replicate several times in vitro. Unfortunately, a number of studies have demonstrated that MSCs from various animals undergo spontaneous transformation when cultured for long terms, posing a limit to this approach. Indeed, transformed MSCs show some of the features of senescent cells, with a progressive shortening of telomers and consequently, cell death (Ahmadbeigi et al., 2011; Ren et al., 2011; He et al., 2014). Such an aging process occurring in MSCs appears to be tissue-specific and has been shown to be regulated by evolutionarily conserved signaling pathways. More recently, a signaling pathway that has shown to be tightly associated to age-related cellular changes is the Wnt/β-catenin signaling cascade (Decarolis et al., 2008; Miyama et al., 2010; Stevens et al., 2010). Wnt/β-catenin signaling plays a functional role as a key regulator of self-renewal and differentiation properties in MSCs. Jeoung et al. (2014) found that activation of the Wnt/β-catenin pathway delays the progression of cellular senescence as shown by the decrease in senescence effectors p53 and phospho-retinoblastoma (pRb), lowered senescence-associated β-galactosidase (SA-β-gal) activity, and increased telomerase activity. In contrast, suppression of the Wnt pathway promoted senescence in MSCs (Jeoung et al., 2014). Hoffmeyer et al. (2012) also showed that Wnt/β-catenin pathway is connected and regulates TERT expression through the interaction with Kruppel-like factor 4 (Klf4), a core component of the pluripotency transcriptional network (a schematic representation is depicted in Figure 2). Unfortunately, to date, the mechanism through which the Wnt/β-catenin signaling pathway regulates age-related neurogenic differentiation in MSCs still remains unclear and needs further investigations.

Influence of MSCs Donor Age on Cellular Therapy
It has been assumed that aging is presumably linked to diminished organ repair capacity due to reduced functionality of MSCs. For this reason, it should be taken into account that the effectiveness of MSCs-based therapies are highly influenced by donor age. There are several studies supporting this concept. It was observed that progressively aging murine BM-MSCs exhibit a decline in MSCs number, proliferation, differentiation, angiogenic and wound healing properties, along with enhanced apoptotic and senescent features (Kretlow et al., 2008; Choudhery et al., 2012a, b). In a study using adipose tissue-derived mesenchymal stem cells (AT-
MSCs) from both young and old donors, it was observed that both were able to form colonies, but AT-MSC from younger donors produce more colonies containing larger numbers of cells and increased proliferative rate than those obtained from older donors (Alt et al., 2012). Moreover, AT-MSCs obtained from aged donors displayed increased senescent features, as indicated by the greater expression levels of p16 and p21 genes, which have been indicated as markers of senescence (Stolzing et al., 2008). In the later study, the expression of SA-β-gal was measured and it was also found at higher levels in aged AT-MSCs cultures, while SOD activity was decreased (Stolzing et al., 2008). It was further identified that MSCs from elderly donors became more granular and developed a more flat and larger morphology at passages 5–6, indicating the appearance of typical morphological signs of replicative senescence (Khan et al., 2009). Recently, in a study conducted on hBM-MSCs from young and old donors used to differentiate and promote neurite outgrowth from dorsal root ganglia neurons (DRGn), Brohlin and coworkers observed that treatment of hBM-MSCs with growth factors induced protein expression of the glial cell marker S100 in cultures from young but not old donors. However, exogenous administration of growth factors enhanced the levels of brain-derived neurotrophic factor (BDNF) and of vascular endothelial growth factor (VEGF) transcripts in both donor cell groups.
and partly recovered stemness properties of MSCs from elderly, supporting the hypothesis stated above. Finally, in the same study it was demonstrated that MSCs co-cultured with DRGn significantly enhanced total neurite length only when obtained from young but not old donors. Moreover, MSCs from young donors maintained their proliferation rate while those from the old ones showed increased population doubling times (Brohlin et al., 2012). These observations suggest that MSCs isolated from either young or old donors may benefit of a combinatorial approach to retain, at least in part, their regenerating properties on neurons. Nevertheless, to date MSCs from young donors are still to be considered the first choice MSCs source to use for CNS repair (Figure 3).

Conclusions
The fact that MSCs can be conveniently obtained from different accessible tissues (such as bone marrow, blood, adipose and dental tissue) and demonstrate neuroprotective effects, immunomodulatory properties and self-migratory activity, makes them an attractive therapeutic tool for potential application in neurodegenerative disorders. However, there are some critical points that still need to be clarified before MSC-based therapy can be adopted in clinical practice. These include the reduced stemness properties of MSCs isolated from elderly or caused by long-term expansion in vitro, which could result in reduced efficacy for regenerative cellular therapy. The complex pathways involved in neurodegenerative disorders, should be evaluated with care, in the attempt to extend the current understanding of the pathogenesis of these diseases and identifying targets for intervention. To be suitable for use in neuroregenerative therapy, the mechanisms that govern the self renewal capacity of MSCs should be characterized in depth. For this purpose, it is proposed that scientific effort should focus more on finding the appropriate microenvironment (culture conditions) that more likely will allow to yield sufficient number of functional MSCs. As previously discussed, a molecular mechanism worthy of attention could be represented by the Wnt/β-catenin signaling pathway, whose involvement in triggering the shift of MSCs towards a senescent phenotype appears to be clear. These findings, together with the
evidences obtained with combinatorial approaches using neuroprotective agents, support the idea that trophic molecules, including some neuropeptides, may elicit a regulatory function on the Wnt signaling cascade, which in turn, could be the key element in controlling MSCs senescence (Jeoung et al., 2014). A similar effect could be achieved by targeting the Wnt/β-catenin directly with Wnt analogues. Alternatively, another critical mechanism to target could be telomere regulation, but this strategy has already reach general consensus, since studies on the mechanisms controlling telomere status and regulation in these cells have progressively gained importance in the last years. In fact, strategies to prevent telomere loss or to increase telomere length of MSCs may prevent or delay degeneration and hence the onset of symptoms in neurodegenerative disorders, improving the results of MSCs-based therapeutic approaches. Finally, a further and reasonable method to expand MSCs validity in therapy could be represented by banking younger adipose tissue for later use. Preservation of MSCs at a younger age, when their biological utility is maximal, could provide a useful source of functional MSCs with full regenerative potential for future applications in regenerative medicine.

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