Potential and pitfalls of stem cell therapy in old age

David Piccin and Cindy M. Morshead

Our increasing understanding of resident stem cell populations in various tissues of the adult body provides promise for the development of cell-based therapies to treat trauma and disease. With the sharp rise in the aging population, the need for effective regenerative medicine strategies for the aged is more important than ever. Yet, the vast majority of research fuelling our understanding of the mechanisms that control stem cell behaviour, and their role in tissue regeneration, is conducted in young animals. Evidence collected in the last several years indicates that, although stem cells remain active into old age, changes in the stem cells and their microenvironments inhibit their regenerative potential. An understanding of both the cell-intrinsic stem cell changes, as well as concomitant changes to the stem cell niche and the systemic environment, are crucial for the development of regenerative medicine strategies that might be successful in aged patients.

Adult stem cells are present in various tissue of the adult body and, in many cases, contribute to tissue regeneration (Mimeault et al., 2007). The ability to recruit these cells in directed regenerative medicine strategies has the potential to revolutionize the treatment of disease and dysfunction, and our increasing understanding of adult stem cell populations is helping to make this potential a reality. With the proportion of people aged over 60 years growing faster than any other age group in almost every country in the world, the need for developing regenerative medicine strategies in the aged population is a high priority. The application of stem cell-based therapies in aged individuals (minimally defined as having exceeded 50% of expected life span) demands a sound knowledge of stem cell features that can ultimately affect their efficacy in contributing to tissue repair.

A loss of tissue-specific stem cells with age is an example of a factor that could potentially limit tissue regeneration. Developing strategies to expand the aged stem cell population by inducing stem cell proliferation and enhancing self-renewal divisions would be crucial to utilizing the cells. Such modulation requires an understanding of the intrinsic changes that occur in aging stem cells, as well as the extrinsic cues that modify their behaviour. The vast majority of stem cell research is performed in young animals and will undoubtedly serve as the foundation for future studies. However, the increased attention to the study of stem cell aging over the last few years attests to the recognized importance of such knowledge. Indeed, several important recent findings highlight both the vast potential for stem cell-mediated regeneration and the need for further studies to augment our ability to promote stem cell-based tissue repair in the aged body.

Stem cell activity persists into old age, but is reduced

The stem cells that are present in young adult tissues persist into old age and retain the ability to proliferate and give rise to new cells. Haematopoietic stem cells (HSCs) are the best studied of all adult stem cell populations, being one of the first bona fide stem cell populations to be identified in adults (Till and McCulloch, 1961). Similar to their young adult counterparts, HSCs derived from old age mice are able to completely repopulate the haematopoietic system of irradiated mice (Morrison et al., 1996) and can serially reconstitute recipient animals, a finding that demonstrates the interesting observation that HSCs have potential life spans that exceed the lifetime of the original host (Harrison, 1972). In the nervous system, neural stem cells (NSCs) are similarly maintained into adulthood, where they continue to generate neural progeny and oligodendroglial bulb interneurons throughout the lifetime of the animal (Tropepe et al., 1997; Enwere et al., 2004). Further, murine muscle stem cell (satellite cell) numbers are maintained in skeletal muscle into old age where they continue to generate muscle fibres in response to injury (Conboy et al., 2005). Indeed, stem cell immortality is considered a sine qua non of stem cell identity and stem cells from many adult systems demonstrate this property in vitro, where they can be passaged a seemingly infinite number of times in optimized culture conditions (Reynolds and Weiss, 1996; Kobari et al., 1998; Rubin, 2002). However, apart from the haematopoietic system, the ability of other tissue-specific aged stem cell populations to perpetually contribute to tissue regeneration in vivo has not been tested, primarily owing to a lack of models that permit serial transplantation.

Active stem and progenitor cells persist into old age but these populations are different from their younger counterparts. There is a significant age-related decrease in the proliferation of NSC progeny in the adult NSC niche lining the ventricular system in the central nervous system (CNS), resulting in reduced olfactory neurogenesis and a loss of fine odour discrimination (Enwere et al., 2004). Moreover, a recent study revealed that, in the hippocampus of the adult rat brain, the proliferation of neurogenic progenitor cells, which are thought to contribute to new memory formation, is also greatly reduced in the aged (Hattiangady and Shetty, 2008). In muscle, where the absolute numbers of satellite cells may be maintained in old-age mice, their ability to contribute to muscle regeneration is severely reduced, resulting in impaired muscle regeneration with age (Conboy et al., 2005; Gopinath and Rando, 2008). And, in the haematopoietic system, HSCs are able to repopulate secondary recipients, however they do so at a lower frequency than their younger counterparts and they are at a

1Department of Surgery, Institute of Medical Science, Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, ON M5S 3E1, Canada
2Author for correspondence (cindi.morshead@utoronto.ca)
competitive disadvantage when mixed with HSCs from a young mouse (Geiger and Van Zant, 2002; Warren and Rossi, 2009). The reasons underlying these fundamental differences are not fully understood, but are the focus of much attention. What is clear is that the reduction in total numbers, along with the reduced proliferation of stem cells and their progeny into old age, has important implications for the development of regenerative medicine strategies. An understanding of the mechanisms that underlie these changes is crucial for the use of these cell populations for regeneration in aged patients.

**Intrinsic and extrinsic factors affecting stem cell behaviour change in old age**

The regulation of stem and progenitor cell kinetics is a complex interaction between extrinsic and cell-intrinsic influences. Extrinsic factors such as changes in the precursor cell niche, signals from the surrounding tissue, and signals derived from distant regions of the body, exert their effects in concert with cell-intrinsic pathways (Fig. 1). The relative importance of changes in these various influences during aging seems to be context and tissue dependent, but patterns appear to be emerging as our understanding grows.

**Cell cycle regulators**

Over the last few years the importance of cell-intrinsic cell cycle regulators in controlling stem cell kinetics during aging has become evident. As with all dividing cells, tight regulation of the progression through the cell cycle is important for regulating the rate of cell division. The G1-S cell cycle transition, and its regulation by the Rb family proteins, is one of the most thoroughly studied of these regulatory mechanisms. Upstream of the Rb family proteins in this regulatory pathway is the cyclin-dependent kinase inhibitor, p19Arf, which is also known to induce cellular senescence (summarised in Fig. 2). Nishino et al. found that, during embryonic development, Hmga2-deficient mice have the same numbers of NSCs as littermate controls, but have fewer NSCs postnatally and into young adulthood as measured by an in vitro colony-forming assay. A deficiency in p16Ink4a and p19Arf (in Hmga2/p16Ink4a/p19Arf triple mutant mice) partially rescued the decrease in NSCs in young adult Hmga2-deficient mice, suggesting that Hmga2 represses p16Ink4a and p19Arf, and that p16Ink4a and p19Arf contribute to the reduced NSC self-renewal that is seen in Hmga2-deficient mice. Consistent with the observation that Hmga2 expression declines with age, and unlike littermate controls, old Hmga2-deficient mice do not have fewer stem cells, revealing that Hmga2 ceases to play a role in stem cell self-renewal in the aged brain. The age-related decrease in Hmga2 levels in old age was shown to be partially mediated by Let-7b, a member of the let-7 microRNA family, which negatively regulates Hmga2 and is upregulated in old age.

Taken together, these findings reveal that stem cell quiescence with age is an exquisitely controlled process and crucial to ensure that stem cells do not become tumorigenic, a risk that increases as stem cells age and stochastically acquire DNA damage (Sharpless and DePinho, 2004; Shenghui et al., 2009). It remains to be determined which factors are responsible for modifying these intrinsic regulators of stem cell division and whether these factors are influenced by injury or disease. However, it is clear that the deliberate decrease in the ability of aged stem cells to self-renew has the potential to profoundly affect stem cell-mediated tissue regeneration.

**Cell damage and telomere shortening**

The accumulation of cellular damage is common to all cells with aging. This damage is associated with exposure to reactive oxygen species (ROS), ionizing radiation, chemical mutagens and, in the case of actively proliferating populations such as stem and progenitor cells, repeated DNA replications. As stem cells are responsible for generating new cells throughout the lifetime of the organism, the DNA repair mechanisms found in all cells are particularly important in this cell fraction (Sharpless and DePinho, 2004). In this regard, the progressive shortening of telomeres (the repetitive sequences found at the end of chromosomes that are necessarily shortened at every DNA division) is an intrinsic change that ultimately restricts the number of divisions that a cell can undergo over its lifetime (Gilson and Geli, 2007). A subset of cells, including stem cells, express telomerase, an enzyme that permits the extension of telomere sequences, thereby avoiding telomere shortening and extending their proliferative life span (Rubin, 2002;
Sharpless and DePinho, 2004; Song et al., 2009). Studies involving the transplantation of HSCs from young mice into the bone marrow of aged mice, and vice versa, determined that the age-related changes in self-renewal capacity were largely cell-intrinsic (Rossi et al., 2005) and, more recently, the role of DNA damage and telomere shortening in this process was determined using transgenic mice deficient in mTERT (telomerase), XPD (important for nucleotide excision repair) or Ku80 (important for non-homologous end-joining) (Rossi et al., 2005; Rossi et al., 2007). Interestingly, there was no change in the number of HSCs between any of the transgenic animals and age-matched (young or old) wild-type animals. However, the ability of the HSCs from old-age mutants to contribute to long-term repopulation in irradiated recipients was completely lost. Perhaps not surprising, the researchers also found that DNA damage accumulated with aging in both the mutant and wild-type HSCs. A similar observation was made in the nervous system, where a reduction in the number of NSCs was observed in telomerase-deficient mice (Terc–/–). Strikingly, changes in the phenotype of NSC progeny were also observed with newly generated neurons exhibiting underdeveloped neuritic arbor (Ferron et al., 2009). Together these data suggest that, although DNA damage and telomere shortening may not play a major role in stem cell homeostasis during aging, these processes may have a significant impact on the ability of the aged stem cells to contribute to tissue regeneration after injury or disease.

Signalling pathways
The extent to which stem cell behaviour is regulated by intrinsic mechanisms versus extrinsic cues, and to what extent these factors are separable, is still debated. These types of changes could be thought of as two sides of the same coin, whereby intrinsic stem cell changes reflect the response of the stem cells to the altered microenvironment, or the intrinsic stem cell changes modify the stem cell response to an unchanged microenvironment. An example of this later change is the downregulation of the epidermal growth factor receptor (EGFR) observed in the neural progenitor population in vivo with aging (Enwere et al., 2004). Signalling through this pathway is important to maintain proliferation of the progenitor cells in the NSC niche. Indeed, the reduction in neurogenesis during aging is concomitant with a decrease in the number of cells expressing components of the pathway. Interestingly, the administration of exogenous epidermal growth factor to the CNS results in only a modest increase in the numbers of progenitor cells within the aged brain, indicative of a change in the ability of the aged cells to respond to the factor. The interplay between intrinsic and extrinsic cues is further demonstrated by the fact that the neural stem cell niche in the aged brain has less tumour necrosis factor \(\alpha\) (TNF\(\alpha\)), the endogenous ligand for EGFR.

**Systemic factors**
Changes in the stem cell environment that occur with aging are well documented and, unquestionably, these extrinsic changes affect stem and progenitor cell behaviour. A concrete example of extrinsic cues modifying stem cell behaviour was illustrated by the work of Conboy et al. (Conboy et al., 2005), who demonstrated that the decline in regeneration potential of muscle stem cells (satellite cells) with aging was the result of changes in the systemic environment (Conboy et al., 2005). Using parabiotic pairings (a shared circulatory system) between young and old mice, they determined that factors in the young circulatory system were able to restore muscle tissue regeneration after injury in old mice. A number of known receptor-ligand interactions within stem cell niches have been shown to modify the behaviour of stem cells in general. For example, the Notch signalling pathway, mediated by the ligand Delta, plays a role in stem cell maintenance (Wilson and Radtke, 2006), and reports suggest that injury-induced upregulation of Delta expression is reduced in the muscles of aging mice (Conboy et al., 2003). Consistent with this finding, Conboy et al. (Conboy et al., 2005) observed enhanced expression of Delta in aged muscle stem cells after injury when exposed to the young environment in the parabiosis model. Further, the increased proliferation of aged muscle stem cells that is observed when cells are cultured in the presence of serum from young animals is significantly reduced when Notch signalling is blocked in vitro. Interestingly, further investigation revealed that Notch signalling leads to suppression of the transforming growth factor \(\beta\) (TGF\(\beta\))-dependent upregulation of cyclin-dependent kinase (CDK) inhibitors, including p16\(^{ink4a}\) and p19\(^{Arf}\), one of the intrinsic regulators that changes with normal aging. Overall, these studies highlight the important role that changes in the systemic environment play in muscle aging. Further studies are needed to elucidate how age-related changes in the environment affect other tissue-specific stem cell populations.

**Important considerations for regenerative medicine in old age**
The lack of a fundamental understanding of the intrinsic and extrinsic changes that occur during aging is one of the most significant hurdles to the development of regenerative medicine strategies that will prove effective in the aging population. There
are a number of significant obstacles that must be considered as this knowledge is pursued. For example, although the cost of housing and caring for animals into old age makes it more cost-effective to perform studies in young animals, researchers must avoid the assumption that findings revealed in young animals will be equally applicable in the aged systems. This pitfall is illustrated in the study of neural regeneration after stroke. In spite of the fact that stroke is more common in aged patients, most studies of stroke are carried out in young animals. In fact, several studies have revealed an impairment in regeneration after brain injury in the aged brain (Popa-Wagner et al., 2009). Young rats recover more quickly than aged rats and undergo complete functional recovery following ischemic cortical injury, whereas aged animals only recover approximately 70% of their pre-stroke motor function (Rosen et al., 2005; Buga et al., 2008). Further highlighting the need to perform studies in appropriate age cohorts is the recent finding that apocynin (a NOX2 inhibitor) administration, a therapy that promotes recovery by reducing oxidative stress following stroke injury in young rats, leads to reduced functional recovery in aged rats (Kelly et al., 2009). Conversely, the study of other age-related diseases such as Alzheimer’s disease or macular degeneration may be more practical in young genetic models of the disease, as the myriad changes in the aged system may obscure the disease pathology. This is illustrated in the study of Alzheimer’s disease, where the pathology is best examined at the earliest stages of the disorder, when the disease manifests as a pure impairment of cognitive function. It is argued that, in such cases, the insights to be gained for therapeutic intervention are greatest prior to the development of the age-related changes that are present with late-stage Alzheimer’s disease brains (Selkoe, 2002).

Although some of the mechanisms that underlie stem cell aging are similar between different systems, the large differences between the tissue-specific stem cells will undoubtedly translate into differences in the way they age. Much of our knowledge about stem cell aging comes from the haematopoietic system, primarily because HSCs are the best characterized of the adult systems. Both HSCs and NSCs divide slowly throughout the lifetime of the animal, continually generating progeny that replace lost cells. As this behaviour is not common to all tissue-specific stem cells, the large pool of literature related to these cell types may be biased towards stem cells that divide similarly, and thus confound our understanding of stem cell aging. On the other end of the spectrum, the importance of systemic factors in stem cell aging primarily comes from work carried out on muscle stem cells. This stem cell pool is only activated in response to stimuli such as injury, so it is beguiling to assume that they will be the most sensitive to changes in systemic factors. Further study will be required to see whether other stem cells respond as substantially to the age-related changes in the systemic environment. Finally, more rapidly dividing stem cells, such as those in the gut, have the additional strain of increased numbers of cell divisions during their lifetime, and an understanding of the mechanisms they use to maintain themselves into old age will probably provide important insight into the stem cell aging process.

The goal of research into regeneration in animal models is the development of regenerative medicine strategies that will be effective in humans. We can take heart from early translational success in the case of haematopoietic reconstitution using bone marrow-derived HSCs, as this now-commonplace human procedure was developed using animal models. Early evidence from the examination of stem cell aging in rodents suggests, however, that they are not necessarily similar to their human counterparts. Studies demonstrating the dichotomy in telomere length in human and mouse longevity (Lorenzini et al., 2009) are a good example of such discrepancies and highlight the importance of proceeding with caution when translating regenerative strategies from animal models to humans.

A variety of stem cell-based strategies are currently being explored for cell replacement. The recruitment of the endogenous tissue-specific stem cells that are already present in the dysfunctioning tissue holds tremendous potential; however, the most commonly applied approach involves stem cell transplantation. Allogeneic transplantation (the transplantation of cells from one patient into another) of HSCs is used in the treatment of genetic disorders such as thalassemia and immunodeficiencies. Autologous transplantation (the transplantation of cells derived from the patient’s own body) of muscle satellite cells has been applied in areas including the treatment of urinary incontinence (Furuta et al., 2007) and Duchenne muscular dystrophy (Torrente et al., 2007). For any stem cell transplantation-based strategy, an important consideration is the age of both the donor tissue and the recipient environment. A recent study using heterochromic parabiotic pairings (linked circulatory systems of old and young adult mice) found that circulating systemic factors from the young mouse increased the engraftment potential of the endogenous HSCs in the aged mice (Mayack et al., 2010) and, further, that this effect was mediated by cells in the aged HSC niche. The ability to modify the aged HSC niche has important implications both for aged donors and aged recipients. Studies examining the engraftment of muscle tissue in aged animals revealed that, despite a striking difference between the histology of aged and young recipients in the short term, ultimately the engraftment and ability of the transplanted muscle stem cells to proliferate and differentiate into muscle are comparable (Shavlakadze et al., 2009). Although this particular analysis demonstrates that stem cell transplants can be very successful in aged recipients, it also reveals that aged recipients will not react identically to younger recipients. Even in young patients and animal models, the outcomes of stem cell transplants vary greatly and this issue will probably be exaggerated in older patients. A deeper understanding of the factors that modify the aged stem cells and their niche will be important for increasing the success of transplants into aged patients.

Approaches that utilize pluripotent stem cells and directed differentiation strategies for transplantation have long been a goal of stem cell research. In addition to the well-documented ethical concerns surrounding the use of pluripotent embryonic stem cells (ESCs), the work is fraught with concerns ranging from the difficulty of controlling differentiation, achieving appropriate integration into specific tissues, the risk of tumorigenesis, and the likelihood of rejection owing to histoincompatibility. Attempts to address the potential for rejection have lead to the development of patient-histocompatible stem cells using techniques such as parthenogenesis or somatic cells nuclear transplant (for a review, see Nehlin and Barington, 2009). Most recently, the discovery that fully differentiated cells can be induced to become cells with...
pluripotent stem cell-like properties, termed induced pluripotent stem cells (iPS cells) (Takahashi and Yamanaka, 2006), has profoundly intensified work in the area. Although this field is in its infancy, the very real possibility exists that converting aged somatic cells to iPS cells may pose problems beyond those that will be encountered when using cells from younger patients. Again, a more comprehensive understanding of the changes that occur in cells during aging is necessary when considering the ultimate success of these strategies.

The future of aging
Early successes in the development of therapeutically relevant manipulations of young adult stem cells attest to the enormous potential that an understanding of the mechanisms that control stem cell behaviour may hold. Examples of such potential strategies include transplantation studies of HSCs that are genetically modified to overexpress the homeobox gene HOXB4, in which there were vastly increased numbers of HSCs in the transplant recipients, with no increased tumour risk (Sauvageau et al., 1995). Similarly, the significant neural regeneration and enhanced functional recovery following growth factor infusion into the NSC niche of the adult rat brain following stroke further attests to the promise for the development of stem cell-based therapies (Kolb et al., 2006). These successes will necessarily provide the backbone upon which future regenerative therapies are built as we consider translating our knowledge into treatment of the aged. Hence, although our current knowledge about aging stem cells provides more promise than pause, it also emphasizes that a first step in the development of regenerative medicine strategies for the aged requires a better understanding of the aging process.

COMPETING INTERESTS
The authors declare no competing financial interests.

REFERENCES


