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The effect of age on stem cell function and utility for therapy

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Abstract

During development, stem cells generate all of the differentiated cells that populate our tissues and organs. Stem cells are also responsible for tissue turnover and repair in adults, and as such, they hold tremendous promise for regenerative therapy. Aging, however, impairs the function of stem cells and is thus a significant roadblock to using stem cells for therapy. Paradoxically, the patients who would benefit the most from regenerative therapies are usually advanced in age. The use of stem cells from young donors or the rejuvenation of aged patient-derived stem cells may represent part of a solution. Nonetheless, the transplantation success of young or rejuvenated stem cells in aged patients is still problematic, since stem cell function is greatly influenced by extrinsic factors that become unsupportive with age. This article briefly reviews how aging impairs stem cell function, and how this has an impact on the use of stem cells for therapy.

Keywords

Stem cells, function, aging, therapy, rejuvenation

Introduction

Stem cells (SCs) are undifferentiated or partially differentiated cells that can, through changes in gene expression, alter their properties to adopt more specialized fates. These gene expression changes are typically implemented through the activity of new transcription factors, whose actions are consolidated by epigenetic modifications. The resulting differentiated cells are those that make up the bulk of the functional tissues and organs of multicellular organisms. In mammals, the so-called embryonic SCs (ESCs) are pluripotent and generate all of the tissue types of the embryo proper, including some more restricted, tissue-specific SCs, also referred to as tissue resident or adult SCs (ASCs). The differentiated cells that form adult tissues are generally replaced, more or less rapidly, by the progeny of these ASCs, most of which are known to persist until death. All of the other cells in the adult body have a relatively limited proliferation potential. As such, ESCs and ASCs form a most convenient cellular source for regenerative therapy.

To remain undifferentiated, all SCs require a key signal from one or more neighbouring specialized cells that form the SC niche. In fact, if a SC is displaced outside of the niche signal range, or if niche signalling is interrupted, the SC embarks on a differentiation route. SCs can divide symmetrically to increase in numbers when they continuously receive the niche signal, while they can also divide asymmetrically to generate one SC and one cell that no longer perceives the niche signal and thus initiates differentiation (reviewed in Morrison and Kimble[1]). An example of primarily asymmetrically dividing SCs are the *Drosophila* germline SCs (GSCs), which divide in a characteristic orientation such that one daughter cell remains attached to the niche and one is born away from the niche and differentiates[2,3]. Depending on the configuration of the niche and the mode of propagation of niche signals, some SCs primarily divide symmetrically and exist as homogeneous proliferative populations. These SCs globally proliferate or differentiate based on the level of niche signal that they distinguish, which is ultimately governed by their distance from the niche. The GSCs of the *Caenorhabditis elegans* nematode provide a clear illustration of symmetrically dividing SCs[1,4,5].

The SC niche was originally defined as the cells, usually located in the proximity of the SCs, that generate the anti-differentiation signal, allowing the SCs to remain undifferentiated and consequently to proliferate and expand in

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numbers[1]. As SCs remain under the influence of niche signalling, growth-stimulating and growth-inhibiting factors then combine from various sources to define SC proliferation rates. While some of these factors may be produced by the niche cells, they may also be released by the differentiating SC progeny, and/or distributed systemically by remote organs. In worms and flies, for example, nutrition leads to insulin/insulin-like growth factor 1 (IGF-1) secretion by the nervous system, leading to systemic activation of insulin/IGF-1 signalling (IIS) and stimulation of GSC proliferation[6–9]. In mice, hair follicle SC proliferation is stimulated by Sonic Hedgehog (Shh), which is secreted by their transit-amplifying progeny[10]. This has led some researchers to expand the niche definition to include any cell that resides in the proximity of SCs and that influences SC biology, including those that regulate their proliferation rhythm[10,11]. This definition would, however, require to better define 'proximity' and to distinguish the type of effect niche signals have on SCs, namely whether they primarily influence SC fate or SC proliferation rates. As the signalling pathways that regulate SC fate are often different from those that regulate SC proliferation[5,12,13], I propose to preserve the original definition of the SC niche and restrict the use of the term 'niche' to the cells (usually located in the proximity of the SCs) that generate an anti-differentiation signal, allowing SCs to remain undifferentiated and able to proliferate (either symmetrically or asymmetrically). Any signal that primarily influences SC proliferation and differentiation rates is then simply termed a growth factor (whether stimulatory or inhibitory) and may originate from any source, including niche cells, the SC's differentiated progeny and remote organs.

This view is somewhat oversimplified, as signals that influence cell proliferation and cell fate are tightly linked. Indeed, signals that are inhibitory to SC proliferation may promote differentiation, and vice versa. For example, the accumulation of p27/Kip1 (a cyclin-dependent kinase inhibitor) in proliferating oligodendrocyte precursors has been suggested to inhibit proliferation and promote differentiation[14]. Similarly, growth factors such as those of the transforming growth factor beta (TGF-β) family are known to influence both SC proliferation and differentiation[15,16]. However, according to this simplified view, for any given SC, its fate is primarily governed by niche signalling, originating from the niche cells, while its proliferation rate is primarily set by the sum of the action of the growth factors it is receiving from any source. Notably, this implies that the effects of systemic growth factors, such as IIS activation, can be modified or even cancelled by mechanisms acting more locally. For example, *C. elegans* GSCs may adopt a nearly quiescent behaviour when there is an over-accumulation of differentiated progeny, despite a fully activated IIS that would otherwise promote rapid proliferation[12,13].

SCs thus respond to the niche and to growth signals by either proliferating, differentiating or remaining quiescent, essentially to ensure that organ and tissue needs are precisely met. Aging, however, progressively introduces a bias in the ability of a SC to respond to tissue demand, such that the SC response takes progressively longer, delaying the tissue replacement/repair process. For example, the healing of a muscle injury, which requires muscle SC function, becomes increasingly lengthy and imperfect in aged mice[17,18]. This is comparable to bone fractures in humans, which have been shown to take much longer to heal in elderly patients[19,20]. Also, the normal turnover of skin cells (dependent on skin SC function) obviously slows down dramatically with aging[21]. I briefly review here the general mechanisms by which aging impairs SC function, and identify the challenges these pose for SC use in regenerative therapy.

Effect of age on stem cell function

The functionality of a SC describes how accurately and efficiently it responds to signals from the niche (or lack thereof) and to growth factors. A young and highly functional SC will efficiently respond to growth factors to either proliferate or remain quiescent when in the niche, or to differentiate when it exits the niche. A young SC will also accurately transmit this functionality to both of its daughter cells when dividing. The general efficiency of most cellular and intercellular processes, however, tends to decline during aging, and this has an impact on SCs. In *C. elegans*, for example, the mitotic index of the GSCs is highest during larval stages and progressively declines during adulthood, even when there is a constant prominent demand for differentiated tissue (oocyte) renewal[12]. The in vitro proliferation of mesenchymal SCs isolated from rats and humans similarly decreases with age[22,23]. In aging rodents, moreover, the proliferation of hypothalamic neural SCs progressively decreases in vivo, such that these cells become depleted in aged mice[24,25]. In this case, neural SC depletion contributes to systemic aging because these SCs release anti-aging exosomal components, which may include microRNAs[26,27]. SC differentiation potential is equally reduced in older animals. For example, hematopoietic SCs (HSCs) tend to fail to differentiate properly in aged mice and have a greater propensity towards the myeloid fate[28–30]. Likewise, mesenchymal SCs isolated from the bone marrow of rats and monkeys show a reduced in vitro differentiation potential[22,31]. Thus, as animals age, the timing, efficiency and accuracy of SC response to growth factors and/or niche signals progressively decline.

Aging impairs SC functionality both cell autonomously and cell non-autonomously (Fig. 1). While aging alters blood composition, it was demonstrated that supplying an aged mouse with blood from a young donor restored the function of many kinds of ASCs[17,32–37]. Although the effects are more pronounced when the animals' circulatory systems are connected through parabiosis, heterochronic blood exchange also has a significant rejuvenating effect on muscle SCs[38]. Thus, systemic factors present in a young animal's blood stimulate SC function and/or factors present in an aged animal's blood impair SC function. More locally acting growth factors, such as those that reflect the demand for SCs' differentiated progeny, may also be produced in aberrant concentrations, or have

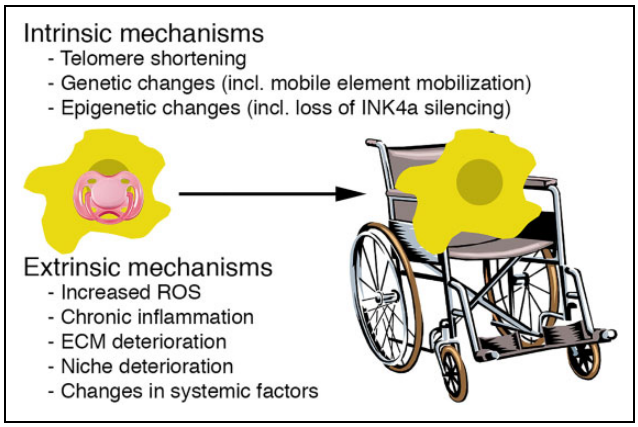

Fig. 1. Intrinsic and extrinsic mechanisms of stem cell aging. SC aging occurs through various cell autonomous (intrinsic) and cell non-autonomous (extrinsic) mechanisms. Refer to the text for details.
ECM: extracellular matrix; ROS: reactive oxygen species.

altered diffusion or transport dynamics, in aged organisms. Like most tissues, the SC niche also deteriorates during aging. In addition to potential perturbation in niche signalling per se, which can mainly perturb SC pool sizes and spatial organization in aged animals[8,39], connective tissue and extracellular matrix deterioration in the SC vicinity can further compromise SC responses to stimulatory or inhibitory signals[40]. Aged tissues are also characterized by increased levels of reactive oxygen species (ROS), causing oxidative damage, which can indirectly impair SC function[41,42]. In particular, ROS are associated with a specific age-induced deterioration of the immune system termed 'inflammaging', which is characterized by a chronic, low-grade inflammation that contributes to the deterioration of SC function[43,44]. Thus, aging of the SC micro- and macro-environment indirectly impairs SC function at multiple levels.

SCs also age cell autonomously, although at the moment it is unclear whether this intrinsic SC aging occurs entirely or partially as a consequence of the deterioration of the SC extrinsic factors mentioned above[44,45]. When SCs are harvested from young animals (i.e. ESCs or GSCs), they can be cultured almost indefinitely in vitro using static culture conditions, yet they are not completely immortal. Telomeres consist of short DNA repeats that cap and protect chromosome ends. In the absence of telomerase, as in somatic cells, the length of these repeats decreases with each round of DNA replication. Despite the continued telomerase expression that characterizes SCs, telomere shortening still occurs in SCs, although at a greatly reduced pace[46–48]. Indeed, a pioneering study demonstrated that although murine GSCs globally retained their proliferative and differentiation potential after 2 years of in vitro culture, their telomeres became significantly shorter[49]. Since SC aging is observed in the absence of external changes in these static in vitro culture conditions, it suggests that SCs do age cell autonomously. Telomere shortening was more recently shown to occur in vivo in aging murine neural SCs[50]. Furthermore, exposure to mutagens and background radiations, as well as the imperfect nature of the DNA replication process, combine to create rare random mutations that accumulate over the lifetime of SCs as they divide[51]. Retrotransposons are mobile DNA elements whose movement may intrinsically contribute to mutation accumulation and aging-induced SC dysfunction[19]. Consistently with this, the efficiency of long interspersed element 1 (L1) retrotransposon repression by SIRT6 was found to decrease with age in neural progenitor cells[52,53]. Finally, characteristic changes have been observed in the aging SC epigenome, such as global increases in the activating H3K4me3 in HSCs and a global decrease in the repressive H3K27me3 mark in muscle SCs[54–58]. Telomere shortening and an altered genome and epigenome thus intrinsically impair aged SCs, while some of these changes likely occur in response to aging at the organismal level in vivo.

Quiescence (a temporary and fully reversible cell cycle arrest) is a normal SC response to the lack of stimulation by growth factors, and may happen for more or less prolonged intervals in any given SC type. Muscle SCs, for example, are quiescent for the major part of an adult's life and are typically reactivated only following muscle injury, to generate new muscle cells[59]. Hair follicle SCs, on the other hand, repeatedly go through short bouts of proliferation, followed by periods of quiescence[11]. Other populations, such as the SCs of the large intestine, are amongst the most solicited in humans, dividing to completely renew the epithelium at least once per week[51,60]. In muscle SCs, it was recently noted that if the epigenetic silencing of the INK4a locus was lost, a phenomenon that increased with age, the cell cycle inhibitor $p16^{INK4a}$ was consequently up-regulated. This caused muscle SCs to undergo senescence upon stimulation by the growth factors that are released upon regenerative pressure[61]. Interestingly, this shift in SC response tended to occur only past a certain age (after 28 months); in geriatric mice. This indicates that an aging threshold is reached in mice around this age, after which muscle SCs tend to become intrinsically and permanently dysfunctional, entering senescence upon further stimulation by growth factors. Consistently with this, transplanting aged muscle SCs into a young environment fails to rescue age-associated phenotypes[62,63]. Therefore, aging perturbs SC function through both intrinsic and extrinsic mechanisms (Fig. 1).

Stem cell rejuvenation

Numerous attempts to return function to aged SCs have shown some degree of success (reviewed in Neves et al.[44]). These include therapeutic agents that were either applied systemically or locally to target SC function, as well as ex vivo rejuvenation procedures followed by transplantation (Fig. 2). Some of the developed experimental settings for systemic rejuvenation treatments, however, such as heterochronic parabiosis, are not readily translatable for therapy in humans[39]. Heterochronic blood exchange may be a more

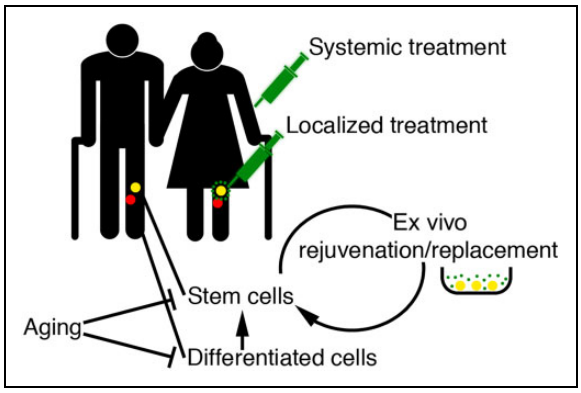

Fig. 2. Effect of aging on stem cells and rejuvenation strategies. Aging impairs SC function both directly (cell autonomously) and indirectly (cell non-autonomously) through impairing the function of differentiated cells. Aged differentiated cells, which include the SC niche and various growth factor-producing cells, offer a leaner support for SC function, which indirectly contributes to intrinsic SC aging. Strategies for regenerative therapies include systemic and localized treatments with rejuvenating factors to directly or indirectly (through restoring the function of SC-supporting differentiated cells) restore SC function. Aged SCs may also be collected from patients, rejuvenated by an in vitro treatment and re-implanted. Such treatment may include pluripotency induction. Alternatively, young SCs collected from the same patient earlier in life, or from a young donor, may be implanted.

practical solution, but in mice, the rejuvenating effects were greatly reduced compared with parabiosis[38]. Nonetheless, a patient-funded clinical trial is being carried out by a start-up called Ambrosia in the USA, where aged patients pay to receive plasma transfusion from donors aged under 25 years[64]. If blood-borne rejuvenating factors could be identified, however, their continual systemic pharmacological administration at optimized therapeutic doses does indeed appear to be a most promising clinical avenue for non-autonomous SC rejuvenation. Successful pharmacological SC rejuvenation examples in the mouse include the systemic administration of interleukin-22 (a cytokine), which restored intestinal SC function[65]. The systemic administration of either oxytocin (a neuropeptide) or Trolox (a vitamin E analogue) was also found to rejuvenate muscle SCs[66,67]. Additional benefits may conceivably be gained through the identification of blood-borne aging factors that impair SC function in aged organisms, followed by their elimination from the blood stream (e.g. affinity filtration, etc.) or inactivation (e.g. monoclonal antibodies). The combination of supplying young positive factors with the removal of aged negative factors may represent the future of the systemic approach to SC rejuvenation.

An alternative and possibly complementary approach whereby $p16^{ink4a}$-positive senescent cells are cleared, either genetically or by a senolytic agent (ABT263), has recently demonstrated promising results by rejuvenating HSCs and muscle SCs in aged mice, and also increasing their healthspan[68–70]. Indeed, senescent cells accumulate with age, and it appears that their presence impairs the function and expansion of healthy SCs[68]. A major downside of all systemic approaches to SC rejuvenation and of their use for therapy is the potential undesired side effects these treatments can have in untargeted tissues. For instance, systemically providing an excess of a growth factor, such as insulin/IGF-1, may stimulate the proliferation and differentiation of many cell populations within an organism, not only the targeted SCs[71–73]. Moreover, as we now know that SC activity is also influenced by local signals, including those emitted by the SCs' differentiated progeny[10,74,75], we also know that these signals can greatly attenuate the impact of systemic growth factors[12]. Thus, systemic approaches to SC rejuvenation, even if some demonstrate sufficient therapeutic efficacy, will likely always necessitate precautions when being used.

Drug delivery for localized therapy is an active area of research (reviewed in Tibbitt et al.[76]) and indeed, the local delivery of SC rejuvenating factors is a promising solution to specifically and more efficiently target a given SC population. Successful examples of localized SC rejuvenation in the mouse include intra-muscular injection of factors to locally reactivate muscle SCs through inhibition of JAK-STAT signalling or activation of β1 integrin signaling[77–79]. Macrophage modulation in the retina by intravitreal injection of mesencephalic astrocyte-derived neurotrophic factor (MANF) was also shown to stimulate retina repair[80]. Finally, topical application of pyridone-6 was found to locally inhibit JAK-STAT signalling and improve hair follicle SC function[81]. Localized delivery should, in principle, be applicable to growth factors that normally act systemically, should their biochemical properties be altered to prevent their diffusion into the blood stream following localized administration. Alternatively, such factors may be distributed systemically, but locally activated or uncaged. Although non-intravenous injections have been used for local SC targeting, this promising avenue is in continuous development and will benefit from state-of-the-art systems that are currently being developed, encompassing nanotechnologies, hydrogels and photo-activation, to name a few[76]. The localized administration or activation of SC rejuvenating factors has the obvious advantage of minimizing the risk for potential unwanted side effects in other tissues, while maximizing the factor's efficacy on the targeted SC population. Localized strategies are anticipated to dominate SC rejuvenating therapy in the near future.

Other successful approaches have relied on ex vivo rejuvenation treatments of SCs isolated from aged patients prior to their re-implantation. Two groups have achieved ex vivo rejuvenation of muscle SCs by exposure to compounds that inhibit p38 MAPK signalling[62,63], while a third group has rejuvenated HSCs ex vivo with a casein treatment that is believed to work by inhibiting Cdc42[28]. Ex vivo rejuvenating treatments provide several obvious advantages over systemic and localized in vivo treatments, offering a highly controlled and accessible environment for manipulating and

treating SCs at optimal drug concentrations without the risk of causing immediate side effects. The downside is a potentially traumatizing sampling and re-implantation of the SCs. This can nonetheless be attenuated if differentiated cells (i.e. skin cells) are sampled instead of the targeted SCs and reprogrammed ex vivo into induced pluripotent SCs (iPSCs) to rejuvenate the cells by erasing age-dependent epigenetic marks[82,83], followed by directed differentiation into the desired SCs or tissue and re-implantation. Although a passage through the pluripotent state is not necessary (direct reprogramming is also possible[84,85]), it induces telomere lengthening due to telomerase reactivation, which contributes to restoring SC functionality[48,86,87]. Obviously, ASCs can themselves be rejuvenated by iPSC technology.

The iPSC strategy, however, also has a limit, since aging suppresses reprogramming to iPSCs[88,89]. Despite this roadblock, one group has been able to derive functional iPSCs from human centenarians[87]. Yet, rejuvenation by iPS reprogramming is incomplete, leaving age-accumulated DNA mutations and incompletely erased epigenetic marks behind, and can even impair specific SC functions[90,91]. For example, the iPS rejuvenation of human mesenchymal SCs led to an incomplete reacquisition of immunomodulatory function[90]. Transplanted iPS-derived cells also commonly form invasive teratocarcinoma-like tumours in mice, yet this may be fully abolished through technical adjustments in the procedure for generation and transplantation of iPSCs. Indeed, a recent study has shown that transgene-free, cMYC-independent reprogramming, along with the elimination of residual iPSCs following differentiation treatment, allowed teratoma-free transplantation of differentiated iPSC progeny in mice[92]. The possibility of using iPSC technology to rejuvenate ASCs and tissues for autologous transplantation in aged patients thus remains a most promising strategy, and more studies are required to better characterize potential imperfections and safety concerns, as well as to identify the means to correct as many of these issues as possible.

Although some of the aging marks may be globally irreversible, such as the randomly accumulated mutations in the nuclear and mitochondrial DNA, the identification of cell types that are less prone to such age-induced changes (i.e. mutations) in aged patients and their use as the starting material for iPSC rejuvenation may offer an interesting avenue. Alternatively, preserved autologous cord blood cells could serve as the starting material for iPSC generation. Finally, it will be advantageous to supplement re-implantation strategies with systemic and/or localized treatments in order to rejuvenate the SC environment and fully support the function of the ex vivo rejuvenated SCs or tissue after their implantation.

Limits to stem cell rejuvenation

The main limit to SC rejuvenation is the risk of cancer. Indeed, cancer cells likely emerge from SCs that, after having been submitted to a natural selection favouring cell proliferation and survival, have progressively transformed into cancer cells[51,93]. All differentiated cell types have a limited proliferative potential, such that they are unlikely to accumulate significant cancer-driving changes[51]. SCs, on the other hand, have a relatively high proliferative potential and persist for a lifetime. They thus have the possibility to accumulate cancer-driving changes due to the imperfect fidelity of DNA replication and of epigenetic transmission.

We may be able to rejuvenate most of the capacities of aged, quiescent SCs, and prevent them from executing (or maybe even revert) senescence, but we are likely unable to erase all of the random genetic and epigenetic changes that a SC will have undergone over its years of existence. The passage through a pluripotent state using iPSC technology has the advantage of resetting the epigenetic marks, but it is not fully accurate[94,95]. Thus, 'resetting' using such methods is imperfect. Therefore, it can be expected that giving back the full proliferative capacity to a SC that still carries a range of inappropriate genetic and epigenetic variations, within a sub-optimal aged differentiated environment, will increase susceptibility to tumorigenic transformation. Even the transplantation of young (thus not having accumulated genetic and epigenetic changes) SCs into an aged individual may be problematic, since the aged person's differentiated cells (including the niche) are still characterized by age-dependent defects, and deficient SC implantation and/or regulation may result. Importantly, while the immune recognition and elimination of cancer cells is recognized as an important defence mechanism against cancer, the function of the immune system also deteriorates with age[96–98]. Yet, in most cases, the benefit of SC rejuvenation therapies may outweigh the relatively small increase in a patient's cancer risk, since, based on multiple studies carried out in mice, significant improvement in a patient's life-quality can potentially be achieved.

Perspectives

While factors that intrinsically and extrinsically cause SC aging are still being identified, along with strategies to reverse this phenomenon, it would appear that a combination of localized treatments over a short duration may be a potentially optimal compromise to achieve efficient tissue repair or rejuvenation while minimizing the potential for the rejuvenated SCs to transform into cancer cells.

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