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\textsuperscript{1}). An example of primarily asymmetrically dividing SCs are the \textit{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\textsuperscript{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 \textit{Caenorhabditis elegans} nematode provide a clear illustration of symmetrically dividing SCs\textsuperscript{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\(^10\). 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\(^{\text{Kip1}}\) (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-\(\beta\)) 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, \textit{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 \textit{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
alteration in aged animals, connective tissue and extracellular matrix deterioration in the SC vicinity can further compromise SC responses to stimulatory or inhibitory signals. Intrinsic mechanisms of SC aging occur through various cell autonomous (intrinsic) and non-autonomous (extrinsic) mechanisms. Refer to the text for details. 

ECM: extracellular matrix; ROS: reactive oxygen species.

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.

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. Heterochronic blood exchange may be a more

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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\textsuperscript{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\textsuperscript{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\textsuperscript{65}. The systemic administration of either oxytocin (a neuropeptide) or Trolox (a vitamin E analogue) was also found to rejuvenate muscle SCs\textsuperscript{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\textsuperscript{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\textsuperscript{68–70}. Indeed, senescent cells accumulate with age, and it appears that their presence impairs the function and expansion of healthy SCs\textsuperscript{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 untreated 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\textsuperscript{71–73}. Moreover, as we now know that SC activity is also influenced by local signals, including those emitted by the SCs’ differentiated progeny\textsuperscript{10,74,75}, we also know that these signals can greatly attenuate the impact of systemic growth factors\textsuperscript{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.\textsuperscript{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 signalling\textsuperscript{77–79}. Macrophage modulation in the retina by intravitreal injection of mesencephalic astrocyte-derived neurotrophic factor (MANF) was also shown to stimulate retina repair\textsuperscript{80}. Finally, topical application of pyridone-6 was found to locally inhibit JAK-STAT signalling and improve hair follicle SC function\textsuperscript{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\textsuperscript{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\textsuperscript{62,63}, while a third group has rejuvenated HSCs ex vivo with a casein treatment that is believed to work by inhibiting Cdc42\textsuperscript{28}. Ex vivo rejuvenation treatments provide several obvious advantages over systemic and localized in vivo treatments, offering a highly controlled and accessible environment for manipulating and
treated 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, 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), it induces telomere shortening due to telomerase reactivation, which contributes to restoring SC functionality. Obviously, ASCs can themselves be rejuvenated by iPSC technology.

The iPSC strategy, however, also has a limit, since aging suppresses reprogramming to iPSCs. Despite this roadblock, one group has been able to derive functional iPSCs from human centenarians. Yet, rejuvenation by iPSC reprogramming is incomplete, leaving age-accumulated DNA mutations and incompletely erased epigenetic marks behind, and can even impair specific SC functions. For example, the iPSC rejuvenation of human mesenchymal SCs led to an incomplete reacquisition of immunomodulatory function. Transplanted iPSC-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. 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 complement 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. All differentiated cell types have a limited proliferative potential, such that they are unlikely to accumulate significant cancer-driving changes. 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. ‘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. 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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**Author contributions**

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References

1. Morrison SJ, Kimble J. Asymmetric and symmetric stem-cell divisions in development and cancer. Nature. 2006;441(7097):1068–1074.

2. Xie T, Spradling AC. A niche maintaining germ line stem cells in the Drosophila ovary. Science. 2000;290(5490):328–330.

3. Yamashita YM, Fuller MT, Jones DL. Signaling in stem cell niches: lessons from the Drosophila germline. J Cell Sci. 2005;118(Pt 4):665–672.

4. Fox PM, Scholl T. Analysis of germline stem cell differentiation following loss of GLP-1 Notch activity in Caenorhabditis elegans. Genetics. 2015;201(1):167–184.

5. Lee C, Sorensen EB, Lynch TR, Kimble J. DAF-18/PTEN locally activates transcription in a probability gradient across the germline stem cell pool. Elife. Epub 5 October 2016. DOI: 10.7554/eLife.18370.

6. LaFever L, Drummond-Barbosa D. Direct control of germline stem cell division and cyst growth by neural insulin in Drosophila. Science. 2005;309(5737):1071–1073.

7. Michaelson D, Korta DZ, Capua Y, Hubbard EJ. Insulin signaling promotes germline proliferation in C. elegans. Development. 2010;137(4):671–680.

8. Narbonne P, Gerhold AR, Maddox PS, Labbé J-C, The C. elegans GSCs: A powerful model for in vivo study of adult stem cell regulation. Int J Stem Cell Res Ther. 2016;3(3):044.

9. Narbonne P, Roy R. Regulation of germline stem cell proliferation downstream of nutrient sensing. Cell Div. 2006;1:29.

10. Hsu YC, Li L, Fuchs E. Transit-amplifying cells orchestrate stem cell activity and tissue regeneration. Cell. 2014;157(4):935–949.

11. Hsu YC, Li L, Fuchs E. Emerging interactions between skin stem cells and their niches. Nat Med. 2014;20(8):847–856.

12. Narbonne P, Maddox PS, Labbé JC. DAF-18/PTEN locally antagonizes insulin signalling to couple germline stem cell proliferation to oocyte needs in C. elegans. Development. 2015;142(24):4230–4241.

13. Narbonne P, Maddox PS, Labbé JC. DAF-18/PTEN signals through AAK-1/AMPK to inhibit MPK-1/MAPK in feedback control of germline stem cell proliferation. PLoS Genet. 2017;13(4):e1006738.

14. Durand B, Gao FB, Raff M. Accumulation of the cyclin-dependent kinase inhibitor p27/Kip1 and the timing of oligodendrocyte differentiation. EMBO J. 1997;16(2):306–317.

15. Lim JY, Kim WH, Kim J, Park SL. Involvement of TGF-beta1 signaling in cardiomyocyte differentiation from P19Cl6 cells. Mol Cells. 2007;24(3):431–436.

16. Watabe T, Miyazono K. Roles of TGF-beta family signaling in stem cell renewal and differentiation. Cell Res. 2009;19(1):103–115.

17. Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature. 2005;433(7027):760–764.

18. McGaechie JK, Grounds MD. Retarded myogenic cell replication in regenerating skeletal muscles of old mice: an autoradiographic study in young and old BALBc and SJL/J mice. Cell Tissue Res. 1995;280(2):277–282.

19. Ahmed AS, Sheng MH, Wasnik S, Baylink DJ, Lau KW. Effect of aging on stem cells. World J Exp Med. 2017;7(1):1–10.

20. Gruber R, Koch H, Doll BA, Tegtmeyer F, Einhorn TA, Hollinger JO. Fracture healing in the elderly patient. Exp Gerontol. 2006;41(11):1080–1093.

21. Farage MA, Miller KW, Elsner P, Maibach Hl. Characteristics of the aging skin. Adv Wound Care (New Rochelle). 2013;2(1):5–10.

22. Bellows CG, Pei W, Jia Y, Heersche JN. Proliferation, differentiation and self-renewal of osteoprogenitors in vertebral cell populations from aged and young female rats. Mech Ageing Dev. 2003;124(6):747–757.

23. Mareschi K, Ferrero I, Rustichelli D, Aschero S, Gammaitoni L, Aglietta M, Madon E, Fagioli F. Expansion of mesenchymal stem cells isolated from pediatric and adult donor bone marrow. J Cell Biochem. 2006;97(4):744–754.

24. de Lucia C, Murphy T, Thuret S. Emerging molecular pathways governing dietary regulation of neural stem cells during aging. Front Physiol. 2017;8:17.

25. Romine J, Gao X, Xu XM, So KF, Chen J. The proliferation of amplifying neural progenitor cells is impaired in the aging brain and restored by the mTOR pathway activation. Neurobiol Aging. 2015;36(4):1716–1726.

26. Dong CM, Wang XL, Wang GM, Zhang WJ, Zhu L, Gao S, Yang DJ, Qin Y, Liang QJ, Chen YL, Deng HT, Ning K, Liang AB, Gao ZL, Xu J. A stress-induced cellular aging model with postnatal neural stem cells. Cell Death Dis. 2014;5:e1116.

27. Zhang Y, Kim MS, Jia B, Yan J, Zuniga-Hertz JP, Han C, Cai D. Hypothalamic stem cells control ageing speed partly through exosomal miRNAs. Nature. 2017;548(7665):52–57.

28. Florian MC, Dörr K, Nielb A, Daria D, Schrezenmeier H, Rojewski M, Filippi MD, Hasenberg A, Gunzer M, Scharfetter-Kochanek K, Zheng Y, Geiger H. Cdc42 activity regulates hematopoietic stem cell aging and rejuvenation. Cell Stem Cell. 2012;10(5):520–530.

29. Janzen V, Forkert R, Fleming HE, Saito Y, Waring MT, Dombkowski DM, Cheng T, DePinho RA, Sharpless NE, Scadden DT. Stem-cell ageing modified by the cyclin-dependent kinase inhibitor p16INK4a. Nature. 2006;443(7110):421–426.

30. Rossi DJ, Bryder D, Zahn JM, Ahlenius H, Sonu R, Wagers AJ, Weissman IL. Cell intrinsic alterations underlie hematopoietic...
stem cell aging. Proc Natl Acad Sci U S A. 2005;102(26): 9194–9199.

31. Yu JM, Wu X, Gimble JM, Guan X, Freitas MA, Bunnell BA. Age-related changes in mesenchymal stem cells derived from rhesus macaque bone marrow. Aging Cell. 2011;10(1):66–79.

32. Baht GS, Silkstone D, Vi L, Nadesan P, Amanti Y, Whetstone H, Wei Q, Alman BA. Exposure to a youthful circulation rejuvenates bone repair through modulation of beta-catenin. Nat Commun. 2015;6:7131.

33. Loffredo FS, Steinhauser ML, Jay SM, Gannon J, Pancoast JR, Baht GS, Silkstone D, Vi L, Nadesan P, Amani Y, Whetstone H, Wei Q, Alman BA. Exposure to a youthful circulation rejuvenates bone repair through modulation of beta-catenin. Nat Commun. 2015;6:7131.

34. Ruckh JM, Zhao JW, Shadrach JL, van Wijngaarden P, Rao TN, Wagers AJ, Franklin RJ. Rejuvenation of regeneration in the aging central nervous system. Cell Stem Cell. 2012;10(1): 96–103.

35. Salpeter SJ, Khalalileh A, Weinberg-Corem N, Ziv O, Glaser B, Dor Y. Systemic regulation of the age-related decline of pancreatic beta-cell replication. Diabetes. 2013;62(8): 2843–2848.

36. Villeda SA, Luo J, Mosher KI, Zou B, Britschgi M, Bieri G, Stan TM, Fainberg N, Ding Z, Eggel A, Lucin KM, Czirr E, Park JS, Couillard-Despres S, Aigner L, Li G, Peskinder ER, Kaye JA, Qu J, Galasko DR, Xie XS, Rando TA, et al. The aging systemic milieu negatively regulates neurogenesis and cognitive function. Nature. 2011;477(7362):90–94.

37. Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosher KI, Luo J, Smith TK, Bieri G, Lin K, Berdnik D, Wabl R, Udeochu J, Wang Y, et al. Aging systemic milieu negatively regulates neurogenesis and cognitive function. Nature. 2011;477(7362):90–94.

38. Rebo J, Mchedipur M, Gathwala R, Causey K, Liu Y, Conboy MJ, Conboy IM. A single heterochronic blood exchange reveals rapid inhibition of multiple tissues by old blood. Nat Commun. 2016;7:13363.

39. Conboy IM, Rando TA. Heterochronic parabiosis for the study of the effects of aging on stem cells and their niches. Cell Cycle. 2012;11(22):2260–2267.

40. Conway A, Schaffer DV. Biophysical regulation of stem cell behavior within the niche. Stem Cell Res Ther. 2012;3(6):50.

41. Geiger H, de Haan G, Florian MC. The ageing haematopoietic system. Aging Cell. 2015;14(5):376–389.

42. Oh J, Lee YD, Wagers AJ. Stem cell aging: mechanisms, regulators and therapeutic opportunities. Nat Med. 2014;20(8): 870–880.

43. Kur J, Wilson C, Passos JF, Oakley F, Correia-Melo C, Greaves L, Saretzki G, Fox C, Lawless C, Anderson R, Hewitt G, Pender SL, Fullard N, Nelson G, Mann J, van de Sluis B, Mann DA, von Zglinicki T. Chronic inflammation induces telomere dysfunction and accelerates ageing in mice. Nat Commun. 2014;2:4172.

44. Neves J, Sousa-Victor P, Jasper H. Rejuvenating strategies for stem cell-based therapies in aging. Cell Stem Cell. 2017;20(2): 161–175.

45. Sacco A, Puri PL. Regulation of muscle satellite cell function in tissue homeostasis and aging. Cell Stem Cell. 2015;16(6): 585–587.

46. Blasco MA. Telomere length, stem cells and aging. Nat Chem Biol. 2007;3(10):640–649.

47. Sun D, Luo M, Jeong M, Rodriguez B, Xia Z, Hannah R, Lovci MT, Morell M, O’Shea KS, Moran JV, Gage FH. L1 retrotransposition in human neural progenitor cells. Nature. 2009;460(7259):1127–1131.

48. Schworer S, Becker F, Feller C, Baht GS, Silkstone D, Vi L, Nadesan P, Amani Y, Whetstone H, Wei Q, Alman BA. Exposure to a youthful circulation rejuvenates bone repair through modulation of beta-catenin. Nat Commun. 2015;6:7131.

49. Kanatsu-Shinohara M, Ogomori K, Iwano T, ◼ Lee J, Kazuki Y, Inoue K, Miki H, Takekushi M, Toyokuni S, Shinkai Y, Oshimura M, Ishino F, Ogura A, Shinohara. Genetic and epigenetic properties of mouse male germline stem cells during long-term culture. Development. 2005;132(18):4155–4163.

50. Rando TA, Zhang W, Li J, Ding Z, Li M, Yi F, Bai R, Wang Y, et al. Aging systemic milieu negatively regulates neurogenesis and cognitive function. Nature. 2011;477(7362):90–94.

51. Van Meter M, Kashyap M, Rezazadeh S, Gundersen V. SIRT6 represses LINE1 retrotransposition in human neural progenitor cells. Nature. 2009;460(7259):1127–1131.

52. Coufal NG, Garcia-Perez JL, Peng GE, Yeo GW, Mu Y, Lovci MT, Morell M, O’Shea KS, Moran JV, Gage FH. L1 retrotransposition in human neural progenitor cells. Nature. 2009;460(7259):1127–1131.

53. Van Meter M, Kashyap M, Rezazadeh S, Gundersen V. SIRT6 represses LINE1 retrotransposition in human neural progenitor cells. Nature. 2009;460(7259):1127–1131.

54. Liu L, Cheung TH, Charville GW, Hurgo BM, Mu Y, Lovci MT, Morell M, O’Shea KS, Moran JV, Gage FH. L1 retrotransposition in human neural progenitor cells. Nature. 2009;460(7259):1127–1131.

55. Van Meter M, Kashyap M, Rezazadeh S, Gundersen V. SIRT6 represses LINE1 retrotransposition in human neural progenitor cells. Nature. 2009;460(7259):1127–1131.

56. Liu L, Cheung TH, Charville GW, Hurgo BM, Mu Y, Lovci MT, Morell M, O’Shea KS, Moran JV, Gage FH. L1 retrotransposition in human neural progenitor cells. Nature. 2009;460(7259):1127–1131.
heterochromatin alterations as a driver of human aging. Science. 2015;348(6239):1160–1163.

59. Conboy IM, Rando TA. The regulation of Notch signaling controls stem cell activation and cell fate determination in postnatal myogenesis. Dev Cell. 2002;3(3):397–409.

60. Frank SA. Dynamics of cancer: Incidence, inheritance, and evolution. Princeton: Princeton University Press; 2007.

61. Sousa-Victor P, Gutarra S, Garcia-Prat L, Rodriguez-Ubreva J, Ortet L, Ruiz-Bonilla V, Jardi M, Ballestar E, Gonzalez S, Serrano AL, Perdiguer E, Muñoz-Cánoves P. Geriatric muscle stem cells switch reversible quiescence into senescence. Nature. 2014;506(7488):316–321.

62. Bernet JD, Doles JD, Hall JK, Kelly Tanaka K, Carter TA, Olvin p38 MAPK signaling underlies a cell-autonomous loss of stem cell self-renewal in skeletal muscle of aged mice. Nat Med. 2014;20(3):265–271.

63. Cosgrove BD, Gilbert PM, Porpiglia E, Mourkioti F, Lee SP, Corbel SY, Llewellyn ME, Delp SL, Blau HM. Rejuvenation of the muscle stem cell population restores strength to injured aged muscles. Nat Med. 2014;20(3):255–264.

64. Kaiser J. BIOMEDICINE. Antiaging trial using young blood stirs concerns. Science. 2016;353(6299):527–528.

65. Lindemans CA, Calafiore M, Mertelsmann AM, O’Connor CA, Rando TA. The regulation of Notch signaling in mice. Nat Med. 2016;22(1):78–83.

66. Elabd C, Cousin W, Upadhyayula P, Chen RY, Choolijian MS, Li J, Kung S, Jiang KP, Conboy IM. Oxytocin is an age-specific circulating hormone that is necessary for muscle maintenance and regeneration. Nat Commun. 2014;5:4082.

67. Garcia-Prat L, Martínez-Vicente M, Perdiguer E, Ortet L, Rodriguez-Ubreva J, Rebollo E, Ruiz-Bonilla V, Gutarra S, Ballestar E, Serrano AL, Sandri M, Muñoz-Cánoves P. Autophagy maintains stemness by preventing senescence. Nature. 2015;528(7583):560–564.

68. Baker DJ, Childs BG, Durik M, Wijers ME, Corbel SY, Llewellyn ME, Delp SL, Blau HM. Rejuvenation of the muscle stem cell population restores strength to injured aged muscles. Nat Med. 2014;20(3):255–264.

69. Baker DJ, Childs BG, Durik M, Wijers ME, Corbel SY, Llewellyn ME, Delp SL, Blau HM. Rejuvenation of the muscle stem cell population restores strength to injured aged muscles. Nat Med. 2014;20(3):255–264.

70. Chang JH, Patel PH, Kohlmaier A, Greneny MO, McEwen DG, Edgar BA. Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the Drosophila midgut. Cell. 2009;137(7):1343–1355.

71. Malaguarnera R, Belfiore A. The emerging role of insulin and insulin-like growth factor signaling in cancer stem cells. Front Endocrinol (Lausanne). 2014;5:10.

72. Nieto-Estevez V, Deferali C, Vicario-Abejon C. IGF-I: A key growth factor that regulates neurogenesis and synaptogenesis from embryonic to adult stages of the brain. Front Neurosci. 2016;10:52.

73. Shim J, Gururaja-Rao S, Banerjee U. Nutritional regulation of stem and progenitor cells in Drosophila. Development. 2013;140(23):4647–4656.

74. Jiang H, Patzl KE, Kohlmaier A, Greneny MO, McEwen DG, Edgar BA. Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the Drosophila midgut. Cell. 2009;137(7):1343–1355.

75. Mondal BC, Mukherjee T, Mandal C, Evans CJ, Sirenko SA, Martinez-Agosto JA, Banerjee U. Interaction between differentiating cell- and niche-derived signals in hematopoietic progenitor maintenance. Cell. 2011;147(7):1589–1600.

76. Tibbett MW, Dahlman JE, Langer R. Emerging frontiers in drug delivery. J Am Chem Soc. 2016;138(3):704–717.

77. Lukjanenko L, Jung MJ, Hegde N, Perrouse-Carrier C, Migliavacca E, Rozo M, Karaz S, Jacot G, Schmidt M, Li L, Metairon S, Raymond F, Lee U, Sizzano F, Wilson DH, Dumont NA, Palini A, Fässler R, Steiner P, Descombes P, Rudnicki MA, Fan CM, et al. Loss of fibronectin from the aged stem cell niche affects the regenerative capacity of skeletal muscle in mice. Nat Med. 2016;22(8):897–905.

78. Price FD, von Maltzahn J, Bentzinger CF, Dumont NA, Yih, Chang NC, Wilson DH, Frenette J, Rudnicki MA. Inhibition of JAK-STAT signaling stimulates adult satellite cell function. Nat Med. 2014;20(10):1174–1181.

79. Rozo M, Li L, Fan CM. Targeting beta1-integrin signaling enhances regeneration in aged and dystrophic muscle in mice. Nat Med. 2016;22(8):889–896.

80. Neves J, Zhu J, Sousa-Victor P, Konjusek M, Riley R, Chew S, Qi Y, Jasper H, Lamba DA. Immune modulation by MANF promotes tissue repair and regenerative success in the retina. Science. 2016;353(6294):aaaf3646.

81. Doles J, Storer M, Cozzuto L, Roma G, Keyes WM. Age-associated inflammation inhibits epidermal stem cell function. Genes Dev. 2012;26(19):2144–2153.

82. Mertens J, Paquola ACM, Ku M, Hatch E, Bohnke L, Ladjevardi S, McGrath S, Campbell B, Lee H, Herdy JR, Gonçalves JT, Toda T, Kim Y, Winkler J, Yao J, Hetzer MW, Gage FH. Directly reprogrammed human neurons retain aging-associated transcriptomic signatures and reveal age-related nucleocytoplasmic defects. Cell Stem Cell. 2015;17(6):705–718.

83. Miller JD, Ganat YM, Kishinevsky S, Bowman RL, Liu B, Tu JT, Toda T, Kim Y, Winkler J, Yao J, Hetzer MW, Gage FH. Directly reprogrammed human neurons retain aging-associated transcriptomic signatures and reveal age-related nucleocytoplasmic defects. Cell Stem Cell. 2015;17(6):705–718.

84. Chambers SM, Studer L. Cell fate plug and play: direct reprogramming and induced pluripotency. Cell. 2011;145(6):827–830.

85. Xu J, Du Y, Deng H. Direct lineage reprogramming: strategies, mechanisms, and applications. Cell Stem Cell. 2015;16(2):119–134.
86. Jaskelioff M, Muller FL, Paik JH, Thomas E, Jiang S, Adams AC, Sahin E, Kost-Alimova M, Protopopov A, Cadinanos J, Horner JW, Flier EM, DePinho RA. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. Nature. 2011;469(7328):102–106.

87. Lapasset L, Milhavet O, Prieur A, Besnard E, Babled A, Ait-Hamou N, Leschik J, Pellestor F, Ramirez JM, De Vos J, Lehmann S, Lemaître JM. Rejuvenating senescent and centenarian human cells by reprogramming through the pluripotent state. Genes Dev. 2011;25(21):2248–2253.

88. Li H, Collado M, Villasante A, Strati K, Ortega S, Camanero M, Blasco MA, Serrano M. The Ink4/Arf locus is a barrier for iPS cell reprogramming. Nature. 2009;460(7259):1136–1139.

89. Soria-Valles C, Osorio FG, Gutierrez-Fernandez A, de Los Angeles A, Bueno C, Menendez P, Martin-Subero JJ, Daley GQ, Lopez-Otin C. NF-kappaB activation impairs somatic cell reprogramming in ageing. Nat Cell Biol. 2015;17(8):1004–1013.

90. El Khatib MM, Ohmine S, Jacobus EJ, Tonne JM, Morsy SG, Holditch SJ, Schreiber CA, Uetsuka K, Fusaki N, Wigle DA, Terzie A, Kudva YC, Ikeda Y. Tumor-free transplantation of patient-derived induced pluripotent stem cell progeny for customized islet regeneration. Stem Cells Transl Med. 2016;5(5):694–702.

91. Solimini NL, Xu Q, Mermel CH, Liang AC, Schlabach MR, Luo J, Burrows AE, Anselmo AN, Bredemeyer AL, Li MZ, Beroukhim R, Meyerson M, Elledge SJ. Recurrent hemizygous deletions in cancers may optimize proliferative potential. Science. 2012;337(6090):104–109.

92. Kim K, Doi A, Wen B, Ng K, Zhao R, Cahan P, Kim J, Aryee MJ, Ji H, Ehrlich LI. Epigenetic memory in induced pluripotent stem cells. Nature. 2010;467(7313):285–290.

93. Lister R, Pelizzola M, Kid SY, Hawkins RD, Nery JR, Hon G, Antosiewicz-Bourget J, O’Malley R, Castanon R, Klugman S, Downes M, Yu R, Stewart R, Ren B, Thomson JA, Evans RM, Ecker JR. Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. Nature. 2011;471(7336):68–73.

94. Mendes F, Domingues C, Rodrigues-Santos P, Abrantes AM, Gonçalves AC, Estrela J, Encarnação J, Pires AS, Laranjo M, Alves V, Teixo R, Sarmento AB, Botelho MF, Rosa MS. The role of immune system exhaustion on cancer cell escape and anti-tumor immune induction after irradiation. Biochim Biophys Acta. 2016;1865(2):168–175.

95. Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ. Natural innate and adaptive immunity to cancer. Annu Rev Immunol. 2011;29:235–271.