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Stem Cells in Aging

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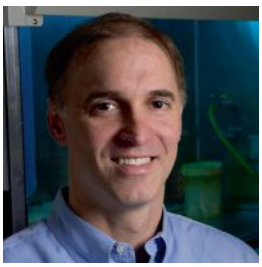
Stem Cells and Aging: What's Next?

We asked 12 leaders in the stem cell and aging fields to share their personal perspectives on the future of the field and the unanswered questions that drive them to work in this exciting area.

Germline Immortality and Aging



Steven E. Artandi
Stanford University

The germline is an immortal lineage in that germline stem cells produce gametes, which generate new embryos through fertilization. Early in development, a new germline is specified, giving rise to stem cells and gametes, which begin the process anew. These germline cycles continue for countless generations in propagating the species, seemingly without the adverse consequences of limitless proliferation. In contrast, the soma executes mundane tasks enabling the organism to eat, metabolize, strategize, and maneuver through its environment. Somatic tissues and the stem cells that maintain them evolved to execute these tasks efficiently, but only through the reproductive life of the animal. Thus, the pronounced aging of the soma may reflect the absence of mechanisms selected for in the germline to facilitate endless renewal without its attendant consequences. Factors that affect genome integrity, such as DNA damage, mutations, and epigenetic alterations are prime candidates to explain potential differences in germline and somatic aging. Differences in relative mutation rates are unclear, but greater fidelity of replication or repair may exist in the germline. It is well recognized that telomeres are efficiently maintained in the human male germline during aging, in contrast to the marked telomere shortening in somatic tissues. The basis for the difference is unknown, but may provide important clues regarding enhanced genome maintenance mechanisms in the germline compared with the soma.

Rejuvenating Muscle

Helen M. Blau
Stanford University

Muscle stem cells (MuSCs) are responsible for the maintenance and regeneration of skeletal muscle mass and are crucial to mobility and quality of life. During aging, the regenerative capacity of MuSCs diminishes and is accompanied by cumulative deleterious systemic, cellular, and metabolic factors. We now know that with advanced age the proportion of MuSCs that are capable of regeneration progressively declines due to cell-intrinsic changes. Deficits include aberrantly active cell signaling pathways, increased expression of senescence markers, and a reduction in stem cell proliferative capacity. Establishing the tipping point for the array of cell-intrinsic defects affords a new therapeutic opportunity and requires a new toolkit. Single-cell analyses will be essential for identifying MuSCs with enhanced regenerative properties. Insights into young and aged MuSCs will arise from time-lapse analyses of genealogic lineage trees that track cellular behavior in response to biochemical and biophysical cues within deconstructed niches. Single-cell mass cytometry and transcriptome analyses will highlight the signaling pathways that go awry and the cell surface molecules that can be used to isolate the most functional subset in the aged MuSC population. This knowledge will guide the quest to identify therapeutic agents that target and expand the residual robustly regenerative MuSCs in aged muscle tissues, thus enabling tissue rejuvenation critical to muscle repair in the elderly. (Photograph by Mari-Carmen Rodriguez, CNIO.)

The Epigenetics of Self-Renewal

Gerald de Haan
European Research Institute for the Biology of Aging

Self-renewal defines stem cells, and if stem cells perfected self-renewal, they would not age. However, most stem cells, including those in the hematopoietic system, lose function over time. Hematopoietic stem cell (HSC) decline likely contributes to age-related hematological disorders, which are rising as the world population ages. So how is self-renewal regulated at the molecular level, and how is this compromised during the aging process? Is stem cell decline caused by an accumulation of random genetic aberrations or by an erosion of epigenetic modifications? The answer is not only of scientific interest but is also relevant for emerging efforts aimed at reversing the aging process. If stem cell aging results from accumulating DNA damage, reversion of stem cell aging seems difficult. In contrast, if stem cell aging occurs as a consequence of altered epigenetic modifications, reversibility may be achievable. Molecules that affect the epigenetic machinery exist with more on the way. Reversing stem cell aging will also depend on whether aging is a cell-intrinsic or cell-extrinsic process. Most data suggest that HSC aging is mostly caused by cell-intrinsic causes, but the final verdict is still out. Indeed, it may be most likely that both pathways are involved. The next decade will reveal, at the single-cell level, how HSCs distribute their epigenetic patterns to their daughters, which of these modifications are crucial for stem cell function, and how they are perturbed during aging.

STEM 1779

Stem Cell Epigenetics in 3D

Hartmut Geiger
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"Epigenetic drifts" are one of the hallmarks of stem cell aging and are an exciting novel area of research. However, how such drifts causally contribute to stem cell aging has remained a puzzle. Novel observations suggest that epigenetic marks and the machineries of epigenetic maintenance also serve a structural function for the genome itself—that is, they regulate 3D interactions between modules that may be separated by considerable linear distance as well as contribute to the overall 3D layout of the genome. An epigenetic understanding of stem cell aging thus moves from linear gene regulation into the 3D world. Recent breakthroughs—for example, the application of the C technologies to single cells, advances in microscopy-based techniques, and improvements in computational modeling—will continue to close our knowledge gap with respect to this fascinating structural function of epigenetics in stem cells. The emerging picture suggests that the spatial organization of the epigenome as a self-organizing and self-perpetuating system uses these epigenetic 3D dynamics to regulate genome function in response to many cues and to propagate cell-fate memory over chronological time and divisions. Studying the 3D perspective of the epigenome with respect to stem cell aging has just begun, and it will certainly unravel novel unexpected mechanisms that might serve as pharmacological targets to improve stem cell and tissue aging in the future.

Aging HSCs and Clonal Collapse

Margaret A. Goodell
Baylor College of Medicine

Turning 50 this year got me thinking about the health of my stem cells and how they will care for me over the next half century. (The melanocyte stem cells on my head having given out years ago, I wish I could peek in my bone marrow.) When young, we are estimated to have about 10,000 hematopoietic stem cells of which ~1,000 actively contribute to the blood. Recent data from multiple groups, including the Ley, Ebert, McCarroll, and Vassiliou labs, suggest that number drops precipitously after the age of 50, such that around 10% of 70-year-olds have a single clone comprising a large fraction of their blood. This finding is not a fluke: together these studies surveyed more than 70,000 individuals across all ages. What drives this clonal hematopoiesis? What are the long-term health implications? What is my own clonal diversity (and should I care)? Mutations in about 20 genes, including *DNMT3A*, *TET2*, and *TP53*, are recurrently associated with clonal collapse, possibly because they confer stem cells with a competitive self-renewal advantage (as my lab suggested for *Dnmt3a* and *Tp53* in mice). Individuals with clonal hematopoiesis have higher all-cause mortality, suggesting an impact on multiple aspects of health. I believe these studies will cause a sea change in our view of healthy aging, and over time they may lead to clonal diversity monitoring and possibly interventions to shift the advantage away from troublesome clones. At the very least, these stem cells will keep me busy for the next few decades.

Countering Stem Cell Aging

Leanne Jones
University of California, Los Angeles

The proper integration of intrinsic factors and extrinsic signals is essential for precise regulation of stem cell behavior and tissue homeostasis. Therefore, it is predicted that defects at all levels (intrinsic, local, and systemic) will contribute to decreased stem cell functionality over time. Significant insights have been gained in characterizing the causes of decreased stem cell function in a range of tissues, but strategies to reverse these changes that will be clinically feasible have yet to be established. With the ultimate goal of restoring stem cell function to sustain healthy tissues, a major challenge will be determining whether targeting one level of stem cell regulation (e.g., reversing extrinsic defects) will be sufficient to alter stem cell aging phenotypes. Although promising results have been demonstrated for some tissues, for others it may be necessary to develop strategies to target both extrinsic and intrinsic mechanisms that will work synergistically to improve stem cell function in an aged individual. Another fundamental question concerns the timing for implementation of such anti-aging strategies—ideally before diseased states are recognized. As we all age at different rates, having a mechanism to predict when an individual may be on the cusp of needing treatment will be beneficial. Therefore, defining a set of genetic and/or biochemical markers to determine an individual's rate of aging early in life also presents an exciting challenge for the future.

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Stem Cells, Aging, and Cancer

Ross L. Levine
Memorial Sloan Kettering Cancer Center

Our understanding of the impact of aging on stem cells and on the susceptibility of stem cells to malignant transformation is rapidly evolving. We have long known that the regenerative capacity of different somatic tissues changes with age and time, and that the risk of developing cancer is highly dependent on age. However, our understanding of the basis for these observations remains incomplete. The recent observation that some elderly subjects have mutant stem cells that undergo clonal expansion has opened our eyes to the idea that stem cells can undergo clonal evolution, even in the absence of overt malignant transformation. How do these mutations, most commonly found in epigenetic regulators, increase self-renewal and stem cell fitness without invariably leading to malignancy? Do these mutations induce a specific epigenetic program, or do they allow for epigenetic instability that then serves as a platform for a specific transcriptional program to evolve over time? Can this process be reversed once it has begun? We and others will spend considerable efforts to understand how clonal evolution in aging stem cells alters self-renewal and sets the stage for malignancy. However, there are many other questions that need to be addressed in our field, including whether the aging process, independent of clonal evolution, can alter the stem cell epigenome and influence the proclivity for transformation. There has never been a better time to ask and investigate these questions.

Quiescence Prescribes Stemness

Pura Muñoz-Cánoves
Pompeu Fabra Univ/ICREA

Adult stem cells sustain tissue regeneration, and their dysfunction with age causes degenerative disease. Over the past decade, heterochronic studies in skeletal muscle have revolutionized the view of the dynamic interactions of muscle stem cells (satellite cells) and the external environment by showing that exposure to a youthful environment can reestablish the regenerative capacity of old muscle, suggesting that stem cell alterations may be tractable. Recent observations have both clarified and complicated this view by showing that cell-intrinsic alterations that cannot be reversed by a youthful environment also contribute to satellite cell decline with aging, particularly at geriatric age. One unexpected finding is that these irreversible cell-intrinsic changes especially affect quiescence, the normal dormancy state that maintains satellite cell stemness, provoking a senescence switch that impairs regenerative functions. Understanding the mechanisms underlying loss of quiescence and acquisition of senescence in geriatric stem cells may help design strategies to delay age-associated tissue decline. I propose that the bona fide functionally reversible quiescent state, rather than a collection of phenotypic markers, is the key for defining stemness in satellite cells. This revised view presents new venues for discerning the function of muscle stem cells in homeostasis, aging, and pathology and for stimulating endogenous stem cells or transplanting exogenous stem cells in therapeutics.

Stem Cells Age… or Do They?

Hans-Reimer Rodewald
German Cancer Research Center

> "Yet who would have thought the old man to have had so much blood in him." —Lady Macbeth, *Hamlet*

Age-dependent decline in stem cell fitness may underlie impaired tissue maintenance and regeneration as well as degenerative diseases and cancer. Stem cells are best studied in their natural environment in vivo; yet, such experiments remain challenging. In the field of hematopoietic stem cells (HSCs), transplantation has been the mainstay of research and clinical applications. Recent experiments in mice uncovered fundamental differences in HSC function when comparing unperturbed in situ HSCs with post-transplantation hematopoiesis. A hallmark of aging HSCs, as deduced from transplantation, was preferential production of myeloid over lymphoid lineage cells. However, modern fate mapping experiments found a drastic "myeloid bias" already in young HSCs that only marginally increased with age. Measurements combined with mathematical modeling revealed normal hematopoietic flow emerging from aged HSCs, leading us to speculate that HSCs could theoretically outlive the mouse itself. This counterintuitive view fits the old man's enormous bloodstain that shook Lady Macbeth. Had she been able to discriminate leukocytes, she would have found healthy granulocytes and monocytes, probably also some naive T and B lymphocytes, born recently from "old" HSCs. Hence, we need to clarify whether "stem cell aging" is the rule or the exception, and to what extent it contributes to lifespan limitations and tissue failing.

STEM 1779

Regenerative Biology of Aging

Amy Wagers
Harvard University

The surprising observation, made 30 years ago in *C. elegans,* that inactivation of even a single gene can be sufficient to slow the aging process and significantly extend lifespan marked a revolution in aging research because it demonstrated that aging can and should be viewed as a biochemically controlled process suitable for molecular dissection, rather than a simple process of "wear and tear." A similar revolution has occurred more recently, with the growing appreciation that not only can the effects of aging be slowed—they may be reversed. Indeed, studies in mice have now demonstrated that certain interventions, including telomerase reactivation, senescent cell ablation, alteration of neuroendocrine signaling, and exposure to age-variant systemic factors can restore "youthful" function in many, distinct aged tissues. The cellular and molecular mediators of these effects are beginning to be uncovered, and they are thus far consistent with the existence of common networks regulating age-associated decline and "rejuvenation" across organ systems. These findings present exciting possibilities for regenerative medicine and also raise the fascinating question: what is rejuvenation in molecular terms? How is cellular function remodeled by these interventions, and do rejuvenated cells truly return to a durable youthful state? Answering this question will resolve essential issues in aging physiology and enable new treatments that can improve human health throughout the lifespan.

DDR in Stem Cells and Progeny

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The maintenance of stem cell functionality is essential for tissue homeostasis, a failure of which leads to organ impairment and premature aging. The aging process occurs in two types of cells: stem cells and postmitotic terminally differentiated somatic cells. The DNA damage response (DDR), including DNA repair, cell-cycle checkpoints, and apoptosis, safeguards the high fidelity of the genetic information amplified in stem cells and dictates cell status, leading to either (1) temporal or permanent arrest of proliferation or (2) apoptosis, both of which ultimately impact tissue homeostasis. Mutations in the DDR cascade cause developmental disorders as well as progeroid syndromes, likely due to the DNA repair defects affecting stem cells during development. Emerging evidence also suggests the commonality of DDR deficits in the decline of adult stem cells and organs during aging.

The key questions facing the field are whether or how the DDR mechanism operates in postmitotic somatic cells and how the genome fitness of stem cells influences the functional competence of their postmitotic (somatic) progenies in adult life. Given that neural impairment is a major aging-associated public health burden, investigating the role of the DDR molecules in the transition of neural stem cells to postmitotic neurons should unravel "novel" functions of the DDR machineries in neural regeneration and degeneration, which may also offer clues on how DDR ultimately prevents tissue impairment and premature aging.

Immortality of Germ Cells

Yukiko Yamashita
University of Michigan, HHMI

Although we consider aging as an inevitable aspect of an organism's life, it is a strange phenomenon in the face of the seemingly immortal nature of germ cells. This paradox represents a fundamental mystery in biology: if germ cells can rejuvenate from one generation to the next, why don't all other somatic cells do so to create an immortal individual that can keep reproducing forever? In other words, if there is a fundamental barrier to achieving immortality in somatic cells, then why can germ cells overcome it?

Interestingly, germ cell production clearly declines with age, implying that there is no magic potion to maintain cells forever (and I do not think that such a decline is purely caused by a decline in gonadal somatic cells). This makes it even more intriguing to ask, "What is really happening in gametogenesis that makes germ cells look as if they are rejuvenated?" The only logical answer seems to be that there is a huge sacrifice/cost in rejuvenating germ cells that is not affordable for somatic cells or is incompatible with their function.

Tackling these questions is not just within the scope of germ cell biology or aging biology: it is a question for all biology. Understanding how germ cells achieve immortality will provide fundamental insight into why we age as well. I would be thrilled to tackle (even bits and pieces of) these questions and/or witness the answer or answers to come into our view. Can I predict when it might happen? No—science is never predictable. (Photo: Michigan Photo Service.)

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Can Metabolic Mechanisms of Stem Cell Maintenance Explain Aging and the Immortal Germline?

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The mechanisms underlying the aging process are not understood. Even tissues endowed with somatic stem cells age while the germline appears immortal. I propose that this paradox may be explained by the pervasive use of glycolysis by somatic stem cells as opposed to the predominance of mitochondrial respiration in gametes.

The question "Why do we age?" is not solved. Understanding the mechanisms driving aging may lead to innovative strategies to increase health span, an effort that would carry enormous human and economic benefit. The fact that many species (typically, though not exclusively, more slowly developing, longer-lived, and larger species) possess somatic stem cells capable of self-renewal and tissue regeneration calls into question why these organisms and their somatic stem cells do age whereas the germline apparently does not. It is also unclear how evolutionary theories of aging that are currently accepted as at least plausible can be reconciled with the biological properties of somatic stem cells. It is proposed here that somatic stem cell maintenance mechanisms lead to preferential accumulation, rather than disposal, of damaged stem cells. On the other hand stringent selection in the germline renders this lineage seemingly immortal. Furthermore, use of glycolysis for ATP production in somatic stem cells as opposed to mitochondrial respiration in the germline suggests that mitochondria play a critical role in stem cell maintenance and gamete selection. This hypothesis is consistent with prevailing evolutionary theories of aging, which I will introduce below, and with a critical role for mitochondria in aging.

Evolutionary Theories of Aging

The most efficacious medical interventions harness naturally evolved processes such as wound repair (surgery), immunological memory (vaccination), endocrine hormones (hormone replacement therapies), and antimicrobials. Understanding the evolutionary rationale of the aging process is therefore critical if we are to prolong life/health span. Several evolutionary theories attempt to explain aging, though none satisfactorily (Kirkwood, 2005). Currently, the most widely accepted explanations are the mutation accumulation and the disposable soma theories (Figure 1). The basis of the mutation accumulation theory is the fact that individuals in the wild typically do not reach old age because of starvation, predation, and exposure (external mortality). This external mortality allows deleterious alleles whose effects occur late in life, and therefore are less subject to purifying selection, to accumulate in a population. The disposable soma theory posits that aging is the consequence of the separation of soma and germline (perhaps driven by external mortality) and is caused by preferential allocation of resources to the germline at the expense of the soma.

Both theories suffer from a lack of defined underlying mechanisms and from observations that have highlighted major exceptions. For example, under the assumptions of the mutation accumulation theory, increased predation should shorten longevity. However, guppies exposed to increased predation did not evolve earlier senescence (Kirkwood, 2005). The disposable soma theory, which focuses mainly on allocation of metabolic resources, may be valid in hermaphroditic species such as *C. elegans*, where the germline makes up a large fraction of the organism. It is unclear however which resources are differentially allocated to the germline at the expense of the soma in sexually dimorphic species, where the metabolic investment in reproduction is lower, in particular in males, which often even have shorter life spans than females.

Maintenance Mechanisms in Somatic Stem Cells

The function of somatic stem cells declines with age (Kirkwood, 2005; Snoeck, 2013), and this decline is at least in part explained by cell-intrinsic mechanisms (Snoeck, 2013). While often viewed as a degenerative condition, aging of somatic stem cells may in fact be a reflection of the pervasive action of protective stem cell maintenance mechanisms that confer differential susceptibility to stress and injury compared to mature cells. Maintenance mechanisms in hematopoietic stem cells (HSCs), the best-characterized postnatal stem cells, include quiescence (associated with the use of the error-prone non-homologous end joining [NHEJ] DNA repair pathway), more active autophagy, and higher resistance to starvation and radiation-induced apoptosis compared to their progeny (Blanpain et al., 2011; Ito and Suda, 2014). Though not as thoroughly investigated, similar maintenance mechanisms are operative in other stem cells. Skin stem cells in the bulge of the hair follicle use NHEJ for DSB repair, have a more transient DNA damage response, and are less radiosensitive than progenitors. Mammary gland stem cells also display radioresistance (Blanpain et al., 2011). There is little or no evidence that stem cell-protective mechanisms fail with age. Radioresistance, starvation resistance, increased autophagy, and the quiescence-associated use of the NHEJ DNA repair pathway suggest that somatic stem cells favor repair, even though it may be incomplete, over disposal by

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damage-induced apoptosis. This preference would lead to stem cell compartments that expand with age, as has been shown for HSCs (reviewed in Snoeck, 2013), and in doing so temporarily maintain their overall function. However, these mechanisms ultimately lead to accumulation of damaged stem cells. Indeed, the repopulation capacity of individual HSCs becomes severely compromised with age (Snoeck, 2013). In contrast, the germline, immortal in a transgenerational sense, uses exactly the inverse mechanism, selection of the fittest gametes (Wallace, 2013). Similarly, asexual reproduction may involve stringent selection of somatic cells.

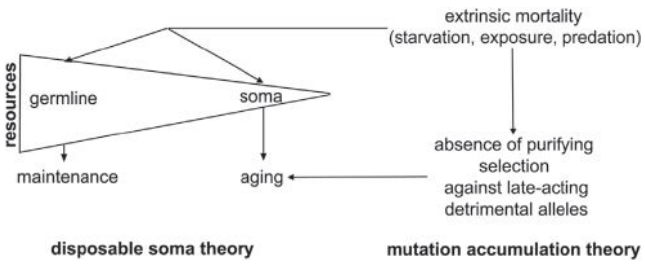

Figure 1. Schematic Representation of the Disposable Soma and the Mutation Accumulation Theories of Aging

Evolved mechanisms must affect reproductive fitness to be subject to selective pressure. Somatic stem cell maintenance mechanisms are likely geared to maximize the probability of reaching reproductive age. Selection, as observed in the germline, would in somatic stem cells lead to rapid attrition and therefore to insufficient tissue maintenance and replacement to be compatible with life up to reproductive age. A similar mechanism of differential maintenance and selection may play a role in the well-established skewing in the differentiation potential of HSCs with age as well. Individual HSCs are heterogeneous with respect to differentiation potential, ranging from predominantly myeloid to predominantly lymphoid. Myeloid-biased HSCs become predominant with age (Snoeck, 2013). While mostly described as a consequence of aging, a shift toward myeloid-biased HSC clones is already observed before birth, suggesting a developmentally regulated mechanism subject to natural selection (Snoeck, 2013). Interpreted in the context of evolved mechanisms of maintenance and selection, stem cell-specific maintenance mechanisms may be more potent in HSCs with predominant myeloid potential, while lymphoid HSCs may be more prone to selection and early attrition. As a consequence, early in life the hematopoietic system generates myeloid cells and long-lived lymphocytes capable of vigorous memory responses, required to protect individuals during reproductive age and to provide passive immunity to their offspring. Later in life long-lived memory lymphocytes persist and myeloid-biased HSCs provide a continuous supply of shorter-lived myeloid cells. What is interpreted as aging may therefore to some extent reflect developmental programming of stem cell maintenance mechanisms aimed at maximizing reproductive fitness.

Role of Mitochondria in Germline Selection and Somatic Stem Cell Maintenance

Mechanisms underlying maintenance and accumulation of damaged somatic stem cells should act in diametrically opposite directions in gametes, where they mediate selection of the fittest to effect reproduction. The differential reliance of somatic stem cells and gametes on mitochondrial respiration for ATP production may be at least one such mechanism (Figure 2).

Oocytes use mitochondrial respiration from pyruvate provided by follicle cells to generate ATP. Strong purifying selection against non-synonymous mutations in mtDNA in genes encoding components of the electron transport chain (ETC) occurs in the female germline, where in humans, only 400 oocytes are ovulated from $>10^6$ proto-oocytes (Wallace, 2013). Sperm cells are continuously produced from spermatogonial stem cells (SSCs). Although SSCs undergo age-related functional decline (Oatley and Brinster, 2012), they produce millions of sperm cells, of which the fittest in terms of motility, capacitation, and zone pellucida binding will fertilize an oocyte. Although sperm motility also requires ATP generated from glycolysis, a sperm's success at fertilizing the oocyte is profoundly affected by mitochondrial dysfunction. In fact, mitochondrial dysfunction and mtDNA mutations, even those that have no discernible consequences in females, are strongly associated with male infertility (Beekman et al., 2014). Stringent selection of sperm during fertilization based on mitochondrial function is therefore likely. Although male mitochondria are lost after fertilization, such a selection mechanism may reduce the transmission of genomic DNA mutations because mitochondrial dysfunction is typically associated with increased oxidative stress.

In contrast to gametes, all somatic stem cells where metabolism has been studied rely predominantly on glycolytic ATP production, while most mature cells use mitochondrial respiration, which is more efficient (Ito and Suda, 2014). Glycolysis in HSCs is typically viewed as an HIF1α-mediated response to the hypoxic BM environment (Ito and Suda, 2014). However, recent evidence suggests that HIF1α is in fact not directly stabilized by hypoxia (except in very severe hypoxia), but by hypoxia-induced reactive oxygen species (ROS) (Sena and Chandel, 2012). These observations run counter to the finding that HSCs are exquisitely sensitive to ROS and exhibit very low ROS levels (Ito and Suda, 2014). Glycolytic ATP production in HSCs may therefore be at least in part hardwired. If gametes are selected based on mitochondrial function, then the pervasive reliance on glycolysis in somatic stem cells, whether hardwired or in response to a hypoxic niche, may achieve the opposite: prevention of selection and maintenance of the stem cell pool, even at the expense of accumulation of subfunctional cells.

A first implication of this idea is that through mechanisms that are as yet unclear, mitochondria may play a key role in somatic stem cell maintenance that is not dependent on ATP production. Indeed, loss of quiescence is typically associated with stem cell depletion and a shift toward OXPHOS (Ito and Suda, 2014) and may be mediated at least to some extent by increased selection and attrition.

A second implication is that mitochondria may be both the driving force and one of the executioners of the aging process. Aging is associated with mitochondrial dysfunction and bioenergetic failure (Wallace, 2013). mtDNA is highly susceptible to mutation and deletion (Wallace, 2013). Absence of mitochondrial selection in somatic stem cells implies imperviousness to accumulation of mitochondrial

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damage and dysfunction. Supporting this idea are the findings that PolgA^{mut} mice, which accumulate mtDNA mutations and display features resembling premature aging, show defects in lymphoid and erythroid lineages, but not in HSCs (Norddahl et al., 2011). The permissiveness of stem cells to mitochondrial damage will contribute to age-associated tissue heteroplasmy, which is defined as the presence of cells with increased mtDNA mutation load. This will affect the function of mature cells and tissues, as these predominantly rely on mitochondrial respiration. Furthermore, defects in the ETC associated with mtDNA damage will increase ROS production. ROS recruit at least some stem cells into the cell cycle and into differentiation (Ito and Suda, 2014). In this way heteroplasmic stem cells could progressively, and even preferentially, participate in tissue remodeling. Preferential recruitment of stem cells that have accumulated mtDNA damage may also contribute to the mechanistically ill-understood phenomenon of the apparent selection for heteroplastic cells in tissues of patients with maternally transmitted mtDNA mutations (Wallace, 2013).

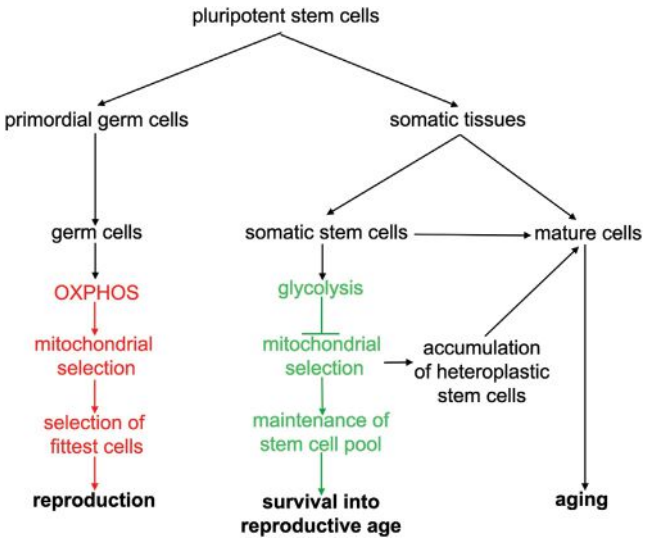

Figure 2. Schematic Illustration of How the Differential Use of Mitochondrial Respiration in Gametes and Somatic Stem Cells Leads to Selection in the Germline and Maintenance in Somatic Stem Cells and Contributes to Tissue Dysfunction and Aging

Tissues with Low Turnover and Interventions that Increase Life Span

In some tissues, such as the CNS, cellular replacement is low to regionally absent. Neurons are extremely reliant on mitochondrial respiration. Interestingly, defects in mitochondrial quality control particularly affect the nervous system (Rugarli and Langer, 2012), suggesting that, in contrast to somatic stem cells, the brain evolved and requires potent mitochondrial quality control mechanisms, which we predict to be less active in somatic stem cells. Such tissues may nevertheless be profoundly affected by age-associated changes in other tissues. For example, aging is characterized by a state of inflammation that may set in motion or perpetuate aging in other tissues. The aging hematopoietic system may itself be a source of inflammatory mediators, produced by senescent memory lymphoid cells, which predominate in the immune system of aged individuals (Snoeck, 2013). Thus, age-associated tissue dysfunction caused by changes in stem cell compartments driven by pervasive stem cell maintenance mechanisms can have systemic consequences, which could in turn also affect stem cell function and the stem cell niche in various tissues.

Several interventions, including caloric restriction, genetic inhibition of nutrient signaling, and sirtuin activation extend life span, in particular in shorter-lived organisms, and may extend health span in long-lived organisms (Kirkwood, 2005). These interventions metabolically rewire cells toward more efficient nutrient utilization and optimize cellular quality control and stress resistance, often at the expense of reproductive capacity. None of these interventions abolishes aging, however. The underlying mechanisms likely evolved to allow organismal maintenance during temporary metabolic stress caused primarily by starvation (Kirkwood, 2005), potentially explaining why the largest effects on life span are observed in the shortest-living organisms.

Stem Cell Maintenance Mechanisms Integrate Theories of Aging

The hypothesis that somatic stem cell maintenance as opposed to germline selection contributes to aging is consistent with the disposable soma theory, which fundamentally posits that the distinction between soma and germline constitutes the origin of the aging process. It would not support the notion that differential allocation of resources between soma and germline mechanistically underlies aging, however. It is also stands to reason that this division of labor arose to maintain the species in the face of external mortality, the driving force of aging according to the mutation accumulation theory. Finally, somatic stem maintenance as a mechanism underlying organismal aging is remarkably consistent with a variant of the mutation accumulation theory, the antagonistic pleiotropy theory, which proposes that mechanisms that provide reproductive or survival benefit early in life are detrimental late in life (Kirkwood, 2005). The idea is testable in a variety of model organisms and stem cells and raises a number of intriguing questions, such as the role of mitochondria in stem cell maintenance and the role and activity of mitochondrial quality control mechanisms in somatic stem cells.

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Regulation of Muscle Satellite Cell Function in Tissue Homeostasis and Aging

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Age-related muscle decline is associated with functional impairment of satellite cells (SCs). Conflicting data suggest dysregulation of cell-extrinsic or -intrinsic factors can independently contribute to such impairment. Here, we emphasize the importance of identifying nodes that integrate these factors into feed-forward circuits, which could provide targets for therapeutic intervention.

Satellite cells (SCs) are tissue-resident muscle stem cells required for postnatal tissue growth and repair through replacement of compromised myofibers. Recent studies have revealed that progressive impairment of SC function correlates with the decline of muscle regenerative potential typically observed during mammalian aging. This functional exhaustion of regenerative potential has been proposed to arise from loss of integrity of the regulatory networks that maintain a quiescent pool of reserve SCs and ensure proper transitions between SC quiescence, activation, and transition into committed progenitors. Quiescent SCs are poised to rapidly respond to microenvironmental cues, such as those provided by extracellular and cellular components of the SC niche, and SC activation occurs as a tightly regulated event in response to muscle injury. The coordinated temporospatial interplay between SCs, differentiated myofibers, and interstitial cellular components of the SC niche is therefore essential for maintaining SC number and function throughout life. Progressive dysregulation of this interplay during aging is emerging as a major cause of loss of SC quiescence.

Experiments utilizing parabiotic conjoining of mice showed that exposure of aged SCs to a youthful environment is sufficient to restore their regenerative potential, indicating a critical role of systemic components in regulating SC function (Brack et al., 2007; Conboy et al., 2005). These experiments revealed a previously unappreciated reversibility of age-associated impairment of SCs by restoring the physiological network of extrinsic cues present in young organisms. More recently, work has identified aberrant activation of several signaling pathways, such as STAT3, p38, FGF2, and canonical Wnt signaling, and a reduction of Notch pathway activity in aged muscles. Interestingly, all of these changes impacted on the transition of SCs to a progenitor stage, leading to impaired control of quiescence and self-renewal (Bernet et al., 2014; Brack et al., 2007; Chakkalakal et al., 2012; Cosgrove et al., 2014; Price et al., 2014; Tierney et al., 2014). Elegant studies from the Brack group provided clear evidence that during aging, increased FGF2 signaling in the aged niche can cause SCs to lose quiescence (Chakkalakal et al., 2012). Subsequent studies linked altered FGF2 signaling with constitutive, aberrant activation of the p38 MAPK pathway, leading to impaired self-renewal of aged SCs (Bernet et al., 2014; Cosgrove et al., 2014). Intriguingly, these two studies performed transplantation assays of aged SCs into younger mice and showed that the aged SC phenotype could not be rescued by a young environment—a finding seemingly in conflict with the conclusions from parabiosis experiments (Brack et al., 2007; Conboy et al., 2005). While this discrepancy may be due to the different experimental settings and assays utilized, we argue that transplanted SCs might be "primed" by the aged organism of derivation and adopt a constitutive, refractory phenotype upon manipulations, such as isolation and transplantation, that are known to artificially activate SCs. Thus, the cell-autonomous resistance to youthful cues observed after transplantation of aged SCs could arise from changes that cannot be erased by exposure to a young environment.

Such a priming mechanism could be mediated by epigenetic changes during aging and in response to extrinsic signals. Consistently, Liu et al. identified transcriptional and epigenetic signatures of SC aging, including loss of bivalency at promoters of developmental genes. Bivalent promoters are simultaneously marked by activatory and repressory marks (H3K4me3 and H3K27me3, respectively). Such promoters are associated with genes poised to be activated during lineage commitment in embryonic stem cells and correlate with stemness in quiescent SCs (Liu et al., 2013). As such, it is possible that progressive loss of bivalent domains compromises quiescence in aging SCs, and such domains might be restored by exposure to youthful cues when SCs are in their native environment. In contrast, physical procedures, such as isolation and transplantation that notoriously lead to SC activation, might impose a resistance to external cues and render these epigenetic changes irreversible.

While cell non-autonomous changes in the aged SC niche may provide the initial trigger ultimately leading to epigenetic dysregulation and compromised SC function, identification of nodes that integrate these disparate cell-intrinsic and -extrinsic signals to sustain the irreversibility of this process might reveal therapeutic targets for anti-aging interventions. The finding that pharmacological blockade of FGF2, p38, and STAT3 signaling, which are aberrantly activated

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in aged SCs, can reverse SC impairment (Bernet et al., 2014; Brack et al., 2007; Chakkalakal et al., 2012; Cosgrove et al., 2014; Price et al., 2014; Tierney et al., 2014) indicates that these pathways control downstream feed-forward circuits that establish and maintain aging-associated changes in SCs. Intriguingly, p38 and STAT3 signaling are essential activators of skeletal myogenesis and promote SC differentiation during regeneration (Price et al., 2014; Tierney et al., 2014), thereby raising the question of how activation of these same signaling pathways can impair SC performance in aged muscles. One possibility is altered cellular distribution of activated pathway components. Presumably, activated signaling components are restricted to committed progeny of SC in young muscles but become uniformly activated in all SCs of old mice. Moreover, changes in the epigenetic landscape in aged SCs might alter chromatin accessibility to downstream signaling pathway effectors, thereby modulating transcriptional output.

The cellular basis underlying the switch from physiological activation of the p38 and STAT3 pathways in young SCs (i.e., by regeneration cues) to age-associated constitutive activation has not yet been conclusively demonstrated. One potential mechanism underlying this switch is the aberrant levels of inflammatory cytokines typically observed in aged organisms. Consistent with extrinsic changes (i.e., the cellular components of the niche, the related secretome, and biomechanical cues) activating a feed-forward mechanism that amplifies age-related events in SCs, exposure to biomaterials that mimic the biomechanical properties of young muscles can rescue the age-related defects of SCs (Cosgrove et al., 2014). A second possibility is that shifts in the heterogeneity of the SC population during aging underlie this switch in activation. Consistently, p38 inhibition and biomechanical cues may only target a subset of the aged SC compartment and change the cellular composition of the SC pool rather than act to reverse the aged phenotype per se.

As mentioned above, cellular senescence is one process associated with the functional decline of aged tissues. While the precise relationship between cellular senescence and aging has not been determined, increasing evidence suggests that senescence can be a "point of no return" at which aging SCs acquire a cell-autonomous phenotype that limits their functional capacity. Of interest, a recent breakthrough from the Munoz-Canoves group has identified a number of senescence-associated features in SCs isolated from over 2-year-old (geriatric) mice. This phenomenon has been termed "geroconversion" and appears to be mediated by de-repression of the cell cycle inhibitor p16 (INK4a)—a hallmark of cellular senescence (Sousa-Victor et al., 2014). This evidence suggests that an altered nuclear landscape in SCs from aged animals might de-regulate gene expression and even alter accessibility of chromatin to certain signaling pathways. In this regard, alterations of histone modifications (i.e., the reduction of genes marked by a "bivalent" chromatin) and histone variants detected in aged SCs (Liu et al., 2013) might alter the physiological response to environmental signals. Thus, in addition to an elevated concentration of upstream extracellular signals in aged tissues, an increased or altered sensitivity to pro-differentiation cues can further contribute to the age-related functional exhaustion of SCs. Interestingly, previous work has shown that p38 signaling can directly control chromatin structure by targeting key components of the chromatin-modifying machinery, including the Polycomb Repressive Complex (PRC2). Disruption of PRC2-mediated gene repression leads to constitutive de-repression of senescence hallmarks, such as p16, and conversion of aged SCs into senescent cells in geriatric mice (Sousa-Victor et al., 2014).

It remains unclear how p38 inhibition could reverse cell cycle arrest associated with cellular senescence (Bernet et al., 2014; Cosgrove et al., 2014), which was previously considered irreversible. A potential explanation arises from the different ages of the mice used in these studies, with p38 inhibition restoring cell cycle activity in pre-senescent, but not senescent, SCs—an effect that has been widely reported in SCs of young mice. The emerging relationships between chromatin structure, signaling pathways, and their potential impact on the irreversible impairment of SCs in geriatric mice deserve future studies.

Although the decline in the regenerative potential of aged SCs is well documented, whether this contributes to reduced homeostatic maintenance and progressive reduction in muscle mass in aged individuals—known as sarcopenia—is still a matter of debate. By utilizing an inducible mouse model to selectively ablate Pax7+ SCs, the Peterson team has recently shown that aging-associated sarcopenia is not affected by depletion of SCs, thereby underscoring the low turnover nature of skeletal muscle and pointing to mature myofibers as the direct cellular targets of this chronic process (Fry et al., 2015). However, an increase in fibrosis in aged muscles was observed in these SC-depleted mice. Thus, while these findings indicate that SCs are not directly involved in aged-associated sarcopenia, they also reveal once again the contribution of SCs to the niche/signaling network that regulates muscle homeostasis. This hypothesis is consistent with a function of SCs not only in tissue repair, but also as sources and targets of secreted or cell-contact-mediated signals for coordinated spatio-temporal regulation of the regenerative niche. Further elucidation of the reciprocal interactions between SCs and the other cell types within the niche will improve our understanding of skeletal muscle homeostasis and identify novel targets for pharmacological interventions to counter age-related muscle loss.

Future Perspectives

The interconnected nature of extrinsic and intrinsic changes occurring in SCs during aging suggests that they are both integral components of a self-amplifying molecular network triggered by age-associated perturbations in the SC niche. It is likely that this network evolves into a cell-autonomous and irreversible cause of SC impairment. Whether this potential scenario corresponds to geroconversion will be an interesting matter for future investigation. Ultimately, elucidating the molecular and epigenetic determinants underlying the interplay between extrinsic and intrinsic factor changes in SCs will reveal the nodal points in this network, and such nodes may be amenable to targeted interventions aimed at interrupting the vicious cycle underlying SC aging. In this context, a significant challenge is posed by the intrinsic heterogeneity of

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SCs. As we move forward, the development of highly sensitive technologies for single-cell analyses will enable us to improve our understanding of SC heterogeneity, how SC populations change with age, and what physiological role this plays in the maintenance of tissue homeostasis. Intriguingly, SC heterogeneity could not only be an intrinsic property of the SC compartment, but may also arise from microenvironmental cues/gradients to which SCs are locally exposed within the tissue. This critical spatial information is lost upon tissue enzymatic digestion and cell isolation, limiting our understanding of SC heterogeneity. The optimization of strategies to perform 3D tissue imaging would enable us to monitor SCs in native tissues, investigate the spatial heterogeneity, and shed light on the relationship between anatomical proximity to specific cell types and establishment of reciprocal functional interactions. These approaches should be complemented with multicolor Cre reporters for clonal lineage tracing in vivo in order to further clarify SC dynamics in living tissues. Finally, understanding the degree to which these animal models of aging and degenerative disease recapitulate human conditions is a major challenge we face. Translational efforts aimed at integrating basic research with patient-oriented studies in the clinic could enable addressing these fundamental questions and further the development of treatments for aging-associated defects in muscle function.

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Stem Cell Aging and Sex: Are We Missing Something?

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Longevity differs between sexes, with females being longer-lived in most mammals, including humans. One hallmark of aging is the functional decline of stem cells. Thus, a key question is whether the aging of stem cells differs between males and females and whether this has consequences for disease and lifespan.

A glance at the list of the human individuals currently living over the age of 110—supercentenarians—reveals a surefire strategy for achieving such exceptional longevity: be female. Out of the 53 living supercentenarians, 51 are female. No other demographic factor comes remotely close to sex in predicting the likelihood of achieving such an advanced age. Sexual dimorphism with respect to longevity is a characteristic of most mammals and has been recorded in human populations since at least the mid-18th century. This dichotomous capacity for resilience has inspired wide-ranging hypotheses to explain the underlying mechanisms. It also raises questions regarding the sexual dimorphism of processes known to sustain tissue regeneration and function throughout life, including adult stem cell renewal. Most adult stem cell populations undergo an age-related decline, leading to dysfunctional tissue homeostasis, which most likely participates in defining the ultimate lifespan of the organism. Interestingly, sex-specific regulation of stem cell populations has been demonstrated for several stem cell types, and it has long been appreciated that many canonical aging pathways exhibit sex specificity. However, despite the seeming interrelationship between sex, stem cell maintenance, and aging, few studies have sought to directly explore the interaction of these three variables. Here we discuss the sexual dimorphism of adult stem cell populations and how processes regulating the aging of stem cells may also be modified by sex.

Sexual Dimorphism of Longevity

Hypotheses to explain the sexual imbalance in longevity have come from divergent fields, including genetics, evolutionary biology, and physiology. One hypothesis is that the single X chromosome of males makes them functionally homozygous at all loci on that chromosome, potentially rendering them more susceptible to deleterious recessive traits. The maternal inheritance of mitochondria could also result in sexual dimorphism in the function of mitochondria, which could in turn impact metabolism and longevity. Evolutionary hypotheses have postulated that the sexes have adapted to be fit for different needs—for example, females put more investment in progeny production and care than males in most species—and that these different adaptive pressures could result in antagonistic selection at longevity associated loci. While these genetic hypotheses are difficult to test experimentally, the evolutionary forces at play have resulted in differential sex-associated phenotypes that may determine lifespan. Most notably, the differential utilization of steroid hormones, estrogen and testosterone (prominent determinants of sex-specific phenotypes), has been proposed to contribute to lifespan. Indeed, studies of human eunuchs (castrated males) have shown that their lifespan is about 14 years longer than non-castrated males (Min et al., 2012). Furthermore, supplementation with estrogen increases the lifespan of male mice specifically (Fontana and Partridge, 2015). However, the mechanisms by which estrogen and testosterone modify lifespan have remained elusive.

Stem Cells in Many Adult Niches Are Regulated in a Sex-Specific Manner

The sex of an organism can modify the behavior of its stem cell populations in a way that may be adaptive. A recent study has shown that hematopoietic stem cells (HSCs) are more abundant and more proliferative in female mice than in male mice, an effect that is dependent on estrogen signaling (Nakada et al., 2014). The dependency on estrogen results in a further increase in HSC number and proliferation during pregnancy (Nakada et al., 2014). A similar paradigm has been described in neural stem cells (NSCs), where estrogen increases the proliferation of NSCs in a transient manner that fluctuates throughout the estrus cycle (Pawluski et al., 2009). NSC proliferation is highest during the proestrus phase of the estrus cycle, when estrogen levels are particularly high. Exogenous administration of estrogen is also sufficient to increase the proliferation of NSCs in vivo (Pawluski et al., 2009). In muscle, the resident stem cells, termed satellite cells (SCs), exhibit greater self-renewal and regenerative capacity in females than in their male counterparts (Deasy et al., 2007). Interestingly, the enhanced regenerative capacity of female SCs does not appear to be related to estrogen signaling as is observed in other stem cell niches (Deasy et al., 2007), suggesting that estrogen signaling is not the sole contributor to differences in stem cell regulation between the sexes. Other studies have shown that females also exhibit increased capacity for rapid

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wound healing and liver regeneration, processes that are likely dependent on resident stem cell populations (Deasy et al., 2007).

Thus, females tend to exhibit increased stem cell self-renewal, regeneration potential, and in some cases, proliferation. However, the question remains: does this tendency toward increased self-renewal in females alter the capacity of stem cells to regenerate tissues throughout aging, and does it influence longevity (Figure 1)?

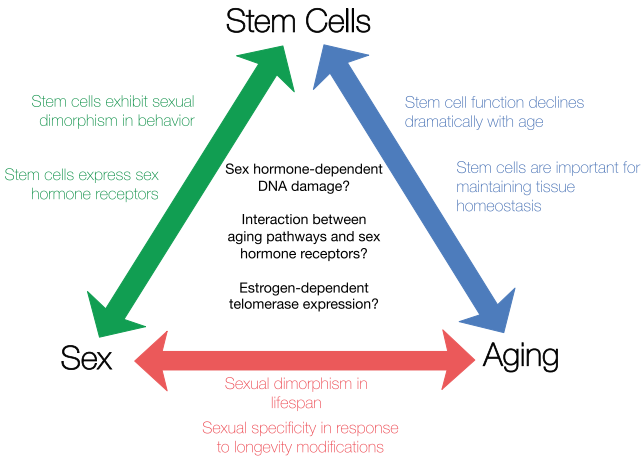

Figure 1. Potential Interactions between Stem Cells, Aging, and Sex
Though interactions between stem cells, aging, and sex have been topics of great interest, the intersection of all three—the effect of sex on the aging of stem cells—has not been well studied. However, several mechanisms could be involved in establishing and perpetuating sexual dimorphism during the aging of stem cells.

The Ability of Stem Cells to Regenerate Tissue Declines with Age and May Be Dependent on Sex

Many stem cell niches experience a decline in self-renewal potential with age (Signer and Morrison, 2013). However, it is unclear whether age-associated changes in stem cells differ between the sexes. Interestingly, many canonical aging pathways have been shown to have a sex-specific effect on lifespan. For example, heterozygous knockout of insulin-like growth factor type 1 receptor (*Igfr1*) increases the lifespan of female mice only (Fontana and Partridge, 2015). In contrast, transgenic overexpression of *Sirt6* increases the lifespan of male mice only (Fontana and Partridge, 2015). What are the effects of sexual differences in aging pathways on stem cells? At this point few studies have addressed this question. However, the limited work in this field does point to potentially fascinating differences between the aging of male and female stem cells. With regards to HSCs, a study of a pair of dizygotic twins—a male and female—with hematopoietic chimerism demonstrated that in the male environment, both genotypically male and female HSCs had shorter telomeres as compared to the same cells in the female (Brüderlein et al., 2008). This finding is consistent with the idea that a female organism provides an environment that is more conducive to sustained self-renewal. This study also suggests an intriguing non-cell-autonomous mechanism of differential aging patterns in male and female stem cells, mediated by the environment of the stem cells. There is undoubtedly a dearth of data addressing the differences in the aging of male and female stem cells, and this will likely be an active area for future investigation.

Potential Mechanisms Linking Stem Cell Aging and Sex

Though the sexual dimorphism of aging stem cells has been largely uncharacterized, there are strong links between sexually dimorphic characteristics—most notably the sex steroid hormones—and classical aging pathways known to be important for stem cell maintenance. These links suggest potential mechanisms that may be involved in generating sex-dependent aging phenotypes in stem cells (Figure 1).

One such mechanism is the sex-specific regulation of DNA damage and reactive oxygen species (ROS). Several recent studies have explored the role of DNA damage and ROS accumulation in stem cell aging, processes which evidence suggests may be modulated by sex. One such study demonstrated that, in muscle, quiescent SCs accumulate DNA damage with age (Sousa-Victor et al., 2014). The accumulation of DNA damage over time in this stem cell population is associated with irreversible senescence (Sousa-Victor et al., 2014), suggesting that DNA damage accumulation can lead to loss of regenerative potential. While the influence of sex cannot be determined in this particular case because the aforementioned study used only males, there is good reason to believe that sex may play a role. Estrogen is known to induce the expression of anti-oxidant genes and reduce ROS, while in contrast testosterone increases oxidative stress. Thus, DNA damage may be bidirectionally regulated by estrogen and testosterone in an oxidative tug-of-war, which may bear consequences for the health and regenerative potential of stem cells throughout the aging process.

Another potential mechanism involves the conserved "pro-longevity" transcription factor FOXO3. FOXO3 is inhibited by insulin signaling and is necessary for increased longevity in insulin signaling mutants (Signer and Morrison, 2013), a pathway that has a greater impact on female than male lifespan (Fontana and Partridge, 2015). Moreover, FOXO3 has been shown to be vitally important for stem cell maintenance in both HSCs and NSCs by preventing premature stem cell exhaustion (Signer and Morrison, 2013). Interestingly, FOXO3 can interact with estrogen receptor (ER-α) (Sisci et al., 2013). This interaction is dependent on estrogen and profoundly alters the cellular effects of FOXO3 (Sisci et al., 2013). These data suggest a fascinating link between a known determinant of stem cell maintenance during aging and sex.

A final prospective mechanism involves telomerase, which is expressed by many stem cell populations and is important for their regenerative potential. Intriguingly, estrogen itself is capable of activating telomerase directly through ER-mediated transcription of TERT, the protein component of the telomerase complex (Kyo et al., 1999). This hints at an intriguing mechanism by which cells in a female environment could delay the aging process through the maintenance of telomeres.

While there are several tantalizing links between known mediators of aging stem cells and sexual dimorphism, their relationships need to be further explored.

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This investigation may lead to new insights about the aging of stem cell populations and the sexual dimorphism of lifespan.

Conclusions

Emerging evidence indicates that adult stem cells in non-sexual tissues are regulated in a sexually dimorphic manner and are responsive to sex hormones. Because stem cell maintenance is important for tissue regeneration throughout life, sex-associated differences in stem cell aging may be associated with sexual dimorphism in lifespan. At this point, however, very limited work has been done to directly address this question.

So what does this mean for the field of stem cell aging? At the very least it should emphasize the importance of controlling for sex in studies in which age is a variable, as most recent work in the field has done. However, beyond that, it suggests a productive line of investigation assessing the effects of sex on the aging of stem cells. It will also be important to understand how sexual dimorphism in aging stem cells predisposes individuals to sex-associated age-related pathologies such as osteoporosis and cardiovascular disease. Furthermore, in any discussion of aging, it is also important to consider not only the lifespan, but also the portion of healthy life or "healthspan" an individual experiences. It is likely that sex plays a role in defining both lifespan and healthspan, and the effects of sex may not be identical for these two variables. As the search continues for ways to ameliorate the aging process and maintain the regenerative capacity of stem cells, let us not forget one of the most effective aging modifiers: sex.

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Programming and Reprogramming Cellular Age in the Era of Induced Pluripotency

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The ability to reprogram adult somatic cells back to pluripotency presents a powerful tool for studying cell-fate identity and modeling human disease. However, the reversal of cellular age during reprogramming results in an embryonic-like state of induced pluripotent stem cells (iPSCs) and their derivatives, which presents specific challenges for modeling late onset disease. This age reset requires novel methods to mimic age-related changes but also offers opportunities for studying cellular rejuvenation in real time. Here, we discuss how iPSC research may transform studies of aging and enable the precise programming of cellular age in parallel to cell-fate specification.

Pluripotent stem cells (PSCs) are characterized by their ability to extensively self-renew and differentiate into all the cell types of the body. During normal development, human pluripotency is restricted to the earliest stages of somatic and germ cell development, stages that can be captured in human embryonic stem cells (ESCs) (Thomson et al., 1998) and embryonic germ cells (EGCs) (Shamblott et al., 1998). The discovery of induced PSCs (iPSCs) (Takahashi et al., 2007; Takahashi and Yamanaka, 2006; Yu et al., 2007) was the realization of a longstanding dream in biology, namely to access pluripotency starting from somatic cells of an adult organism. Human iPSC technology has opened up new frontiers in regenerative medicine and human disease modeling. Protocols for the directed differentiation of human PSCs (hPSCs) have been developed to generate an increasingly broad repertoire of differentiated human lineages, and a future is foreseeable wherein any human cell type can be generated in vitro—on demand and at scale. Although technologies for the programming and reprogramming of cell fate have evolved rapidly, our ability to control the maturation state and age of resulting pluripotent-derived lineages remains rudimentary at best. In fact, there is general agreement that human-pluripotent-derived lineages exhibit the properties of fetal-stage cells such as in the case of hPSC-derived neural, cardiac, or pancreatic lineages. Importantly, such fetal-like properties are observed in iPSC-derived lineages independent of the age of the initial somatic cell donor. The embryonic-like nature of hPSC-derivatives represents a potential barrier to the use of PSCs, which motivates the development of strategies for directing cellular age in vitro, in particular for applications in human disease modeling. On the other hand, those findings raise the intriguing question whether the reprogramming process resets not only cell fate (i.e., from specified to pluripotent) but also the chronological age characteristic of the donor cell population.

Here, we will discuss recent studies that address questions of age in pluripotent stem cells. Those include both efforts to study the apparent rejuvenation process during reprogramming as well as the development of techniques to artificially induce age in iPSC-derived lineages for modeling late-onset disorders. The long-term goal is to reliably program and re-program cellular age independently of cell fate and thereby recrate specific cell types of any age (e.g., 80-year-old neurons, 20-year-old pancreatic cells, or 40-year-old heart cells).

Current Strategies for Studying Aging

According to the World Health Organization, global life expectancy will increase from 48 years of age in 1950 to 73 years by 2025 (http://www.who.int/whr/1998/en/whr98_en.pdf?ua=1), and, in many developed countries, the average life expectancy is already >80 years and rising. The associated worldwide increase in the incidence of age-related disorders such as Alzheimer's (AD) and Parkinson's disease (PD) is expected to cause enormous social, economic, and medical challenges. The mounting problem of an aging society has triggered a race to find novel strategies to treat age-related disorders. A more radical proposition is the search of the "youth elixir," hence the development of techniques that would actively rejuvenate the human body. However, if such strategies were to succeed, they would likely further extend overall human lifespan with unknown consequences for society. In fact, the pursuit of preventing human aging has been touted by some as "egocentric efforts of rich people to live longer" (http://www.reddit.com/r/IAmA/comments/2tzjp7/hi_reddit_im_bill_gates_and_im_back_for_my_third/). On the other hand, anyone who has witnessed the suffering of loved ones can understand the motivation for treating or even better preventing serious age-related disorders and achieving longer and healthier lives. Independently of whether such efforts are considered as "chasing immortality" or simply as addressing the emerging challenge of potentially billions of elderly people facing aging-related consequences, the question of age and longevity is a fascinating scientific problem. The main challenge for tackling this issue is the identification of a unanimously accepted cause of aging.

Modern theories of aging can be classified into programmed versus damage or error-induced mechanisms. Theories of programmed aging argue for a developmentally controlled program that drives aging through the regulation of tissue homeostasis as well as repair and defense responses. In contrast, damage- or error-induced aging theories emphasize the temporal or stress-induced accumulation of damage caused by reactive oxygen species (ROS), cross-linked macromolecules,