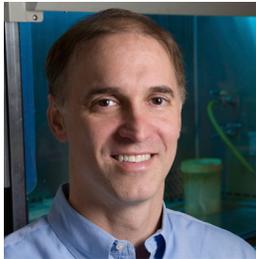


Stem Cells and Aging: What's Next?

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 Amparo Garrido.)

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 Cell Epigenetics in 3D



Hartmut Geiger
University of Ulm/CCHMC,
Cincinnati

“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.

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.

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



Zhao-Qi Wang
Leibniz Institute for Age
Research (FLI), Jena

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.)