WE summarize here the fruitful discussion that occurred on a wide variety of topics during the session on stem cell aging.

SOMATIC STEM CELL AGING

Do stem cells age; and if they do, how can these changes be reliably identified?

Most attendees were of the view that a decline in the function of tissue-specific somatic stem cells is likely to play an important role in human aging. Results from several mammalian systems were discussed including stem cells of the adult bone marrow, brain, intestine, and muscle. Also, there was a brief discussion of what should constitute a stem cell for the purposes of this discussion, for example, cells like pancreatic β cells, muscle stem cells, and memory T cells appear to share some important features with more classically defined stem cells (eg, an ability to self-renew in adult organisms), but which lack the capacity for broad multilineage differentiation thought to be the sine qua non of a “true” stem cell. Although some aspects of mammalian aging may be shared among such long-term, self-renewing cells with aging, a consensus view emerged that the mechanism and functional consequences of aging are likely to differ markedly by tissue and cell type. For example, transplantable and cell-intrinsic hematopoietic stem cell (HSC) function have been identified by several investigators (1,2), and these defects seem to be increased by stimuli that attenuate DNA repair. In contrast, work of Conboy and colleagues (3) suggests that significant aspects of muscle stem cell aging are cell extrinsic and rather reflect changes in the stem cell milieu (or “niche”; see following).

Potential causes of cell-intrinsic aging were discussed. The role of tumor suppressor induction (p53 (4) and p16\(^{INK4a}\) (5,6)) in response to cumulative DNA damage, telomere dysfunction, and other stresses were considered important. These alterations may in turn reflect changes in stem cell chromatin structure, for example, the role of Polycomb group proteins in regulating the \(INK4A/ARF\) locus was discussed as a potential contributor to stem cell aging (7). Along these lines, several of the discussants indicated that studies of epigenetic changes, presumably using both candidate approaches and epigenome-wide genomic approaches, should be priorities for future study (see also article by Kahn & Fraga, [8]). Also, an enhanced understanding of stem cell aging was identified as a critical need to provide reliable biomarkers of the process. Potentially useful biomarkers might be changes in chromatin status, changes in gene expression, or changes in measurable macromolecules (eg, telomeres, DNA mutations, protein modifications).

INTRINSIC VERSUS EXTRINSIC STEM CELL AGING

What are the roles of stem cell aging (cell intrinsic) and niche aging (cell extrinsic) in the decline of tissue homeostasis in aging and age-dependent risk of disease?

The relative contributions of niche versus cell-intrinsic aging were discussed. Several discussants expressed the view that this question may be tissue specific and also that there may be contributions of both types of effects within a given tissue with aging. For example, the work of Ju and associates was (9) discussed showing that animals harboring dysfunctional telomeres have intrinsic functional defects of the HSC as well as extrinsic defects in the bone marrow niche. As changes to the niche (eg, deficiency of some factor that enhances stem cell function) might be particularly tractable to therapy, understanding which features of stem cell functional decline are niche dependent was considered a high-priority question. Several participants wondered if there could be small-molecule approaches to revert niche aging (eg, replacement of missing hormones or inhibitors to block signaling induced by age-promoting ligands in the milieu). Likewise, this discussion led to interest in the topic of whether heretofore unrecognized cell-intrinsic aging could be hampering ex vivo efforts at organogenesis and tissue engineering.
A Possible Need for New Model Systems

What are good approaches to study tissue homeostasis with aging in the absence of injury or regeneration?

A particular frustration of discussants was the lack of consensus on validated models for the role of stem cells in aging. As mentioned, the mechanisms of aging may differ among somatic stem cell fractions (e.g., intestine vs muscle vs HSC), and therefore, there was concern as to how generalizable results from one of these systems would be to stem cell aging as a whole. In particular, several discussants worried that the experimental tractability of the HSC system has placed undue emphasis on results from this model, whereas the mechanism of stem cell aging of the gut or skin may be very different. Thus, new models may be needed to address such questions as whether the cell-intrinsic aging of constantly proliferating stem cells (e.g., gut) is different from the aging of stem cells that are largely quiescent (e.g., the HSC) or activated only in response to stimuli (e.g., muscle satellite cells).

Moreover, concern was voiced in two additional areas regarding model systems: the reliance on “progeroid” mice and humans to inform more general conclusions about mammalian aging, and the frequent need to injure or damage tissues (e.g., using transplantation, ionizing radiation, damaging toxins) for regenerative studies. Some discussants voiced the belief that results from such systems may be systematically biased in nonobvious ways. Approaches to study stem cell function with aging in the absence of reliance on germline genetic mutations that bias toward “premature aging” or severe disruptions of normal tissue homeostasis were discussed. It was pointed out that recent advances in mouse genetics using Cre-lox systems and other means of somatic recombination obviate some of the need for globally damaging events and also allow for lineage tracing and fate mapping in the absence of injury and regeneration models. Additionally, induced pluripotency–based systems may provide new human and murine systems for these lines of research (see following).

A great deal of interest was also expressed in the development of non–mammalian models to allow for the study of stem cells (e.g., fish, planaria, hydra; see also article by Austad, [10]). Although it was pointed out that some of these models may offer significant advantages over rodent systems, some important drawbacks of these systems were also acknowledged. In particular, some of these models are less well characterized in genetic terms, and in some cases, crucial reagents and techniques for studying them are lacking. For this reason, particular note was made of the recent discovery of somatic stem cells in the Drosophila intestine (11,12), a system that is well characterized and experimentally tractable.

Induced Pluripotent Stem Cells, Nuclear Transfer, and Human Aging

What is the significance of induced pluripotent stem (iPS) cells (and other new technologies) for generating valuable research resources for aging investigations and what do they tell us about the aging process itself?

Patient-specific disease-expressing pluripotent stem cells (PSCs) hold great promise as research resources for both understanding diseases of the elderly patients and understanding the aging process itself. The potential uses of these specialty lines include the ability to obtain skin fibroblasts, for example, from patients with early-onset Alzheimer’s or Parkinson’s diseases, as well as their unaffected identical twins or siblings, and then after differentiation in vitro perform “omic” comparisons to decipher the mechanisms of disease directly from the affected patient. In another use, cell lines from frail patients could be contrasted with cells from healthy aged patients. Also, cells from young individuals could be compared with cells from older people to ferret out information regarding fundamental aspects of aging mechanisms.

While PSCs are now routinely established from mice using nuclear transfer or cloning technologies (13), “therapeutic cloning” has not yet succeeded in humans, although it has been reported with rhesus monkeys. Recent breakthroughs in “induced pluripotency” (iPS; 14, 15) have transformed the field because, for the first time, fibroblasts can be “reprogrammed” into their pluripotent states. In the case of mice, these iPS cells will contribute to offspring and are transmitted through the germline—compelling evidence for their pluripotency. Although the first reports used potentially dangerous oncogene inserts, newer approaches are avoiding these likely problems (16, 17).

These research tools immediately force questions as to the differences between old and young cells, and whether fibroblasts or other cells from the elderly population will be refractory to “reprogramming.” Another new breakthrough is the ability to directly differentiate cells from one adult state into another (e.g., exocrine pancreas into endocrine pancreas) (18). If it is possible to “transdifferentiate” differentiated cells, might it also be possible, using the appropriate transcription factors, to reverse the aged phenotype of cells or tissues and in effect reverse the ravages of aging? Research using human cells as well as appropriate non–human primates and large animal species should be encouraged.

Germ Cells and Reproductive Aging Differences Between the Sexes

Do the specialized class of stem cells—the germ cells—age and why are there differences between reproductive aging in women versus men?

The aging of oocytes within the ovaries of premenopausal women and female mammals is an extraordinary example of cellular aging separate from aging within the entire organism. Recently, through the development of powerful male germ cell transplantation procedures, it has been possible to ask whether germ cells in men and male mammals also age. Transplantation of male germ cells from an aged individual into the sterile testes of a young rodent has addressed the
question of the potential aging of spermatogenic lineages (19). After about six or seven generations, aging effects are prominent, which now enable in-depth studies as to whether environmental factors, such as caloric restriction, could delay these processes. Finally, the interconvertibility of “immortal” germ cells and “mortal” somatic cells has again been undermined. Conrad and associates (20) just reported on the derivations of PSCs from testicular biopsies. These lines of research are important as biologists grapple with the fundamental concepts in aging and mortality.

**Future Directions**

Based on the summarized discussions of this session, a few areas were viewed as appropriate for future research in stem cell aging:

1. Implications of new embryonic stem cell research and induced pluripotency on aging and regeneration.
2. Concerted effort to analyze the intrinsic “lesion” (DNA damage, changes in epigenome, etc.) in somatic stem cells with aging by tissue.
3. Concerted effort to optimize and standardize uniform model systems (including in vitro systems) to study stem cell aging, and the development of new model systems to facilitate our understanding of stem cell aging.
4. Given its therapeutic tractability, a focus on discerning which aspects of somatic stem cell aging result from changes in the niche versus stem cell intrinsic changes.

It was felt that addressing these topics would allow for a better understanding of how age-dependent changes in stem cell function might contribute to human aging.

**Acknowledgments**

The authors thank all the participants in this very lively discussion that occurred within the context of the Biology of Aging Summit in September 2008.

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