The Decay of Stem Cell Nourishment at the Niche

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Abstract

One of the main features of human aging is the loss of adult stem cell homeostasis. Organs that are very dependent on adult stem cells show increased susceptibility to aging, particularly organs that present a vascular stem cell niche. Reduced regenerative capacity in tissues correlates with reduced stem cell function, which parallels a loss of microvascular density (rarefaction) and plasticity. Moreover, the age-related loss of microvascular plasticity and rarefaction has significance beyond metabolic support for tissues because stem cell niches are regulated co-ordinately with the vascular cells. In addition, microvascular rarefaction is related to increased inflammatory signals that may negatively regulate the stem cell population. Thus, the processes of microvascular rarefaction, adult stem cell dysfunction, and inflammation underlie the cycle of physiological decline that we call aging. Observations from new mouse models and humans are discussed here to support the vascular aging theory. We develop a novel theory to explain the complexity of aging in mammals and perhaps in other organisms. The connection between vascular endothelial tissue and organismal aging provides a potential evolutionary conserved mechanism that is an ideal target for the development of therapies to prevent or delay age-related processes in humans.

Aging, The Final Frontier

THE PHYSIOLOGICAL CHANGES ASSOCIATED WITH AGING are evident in almost all living creatures. Within the evolutionary diversity of life, aging is generally considered a progressive, functional loss that leads to decline of fertility, increased susceptibility to disease and tissue dysfunction, and increased risk of mortality.^{1–3} Thus, aging is associated with a gradual loss of homeostatic mechanisms that maintain cellular self-renewal and the active function of adult tissues. A major challenge of aging research has been to distinguish the causes of cellular and tissue aging from the myriad of changes that accompany it.

Aging Is Not Tamper Resistant

Although aging seems to be an irreversible process that culminates with death of the organism, several observations and experimental manipulations suggest that life span itself can be modulated. To date, caloric restriction (CR) is the only non-genetic intervention that has been shown to expand life span consistently in all living creatures tested. Limiting the amount of calories taken delays the progressive functional loss and increases life expectancy.⁴ Organisms subjected to CR display common characteristics that have been established as biomarkers of aging. Longevity in non-CR humans

correlates with biomarkers such as low circulating insulin levels, lower body temperature, and maintenance of dehydroepiandrosterone levels.⁵ The insulin/insulin-like growth factor-1 (IGF-I) signaling (IIS) pathway constitutes an evolutionarily conserved mechanism of longevity from yeast to humans.^{6,7} Genetic and environmental manipulation of the IIS pathway has been shown to extend life span of model organisms such as the nematode worm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and laboratory mice.^{8,9} As an example, alterations to the mammalian target of rapamycin (mTOR), the insulin receptor, or the energy-sensing pathways involving 5'-adenosine monophosphate-activated protein kinase (AMPK) have all been shown to extend life span in animal models.^{10–13} It is noteworthy that genetic studies of the human population have revealed that functional mutations in the IGF receptor correlate highly with centenarians.¹⁴ Similarly, genetic variations that reduce IIS correlate with long-lived humans.¹⁵

Adult Cells Can By-Pass Death and Start a New Aging Cycle

Despite the inexorable process of aging, the aging clock in nature restarts after each life cycle. The reprogramming process, which is so central to fertilization, can be simulated experimentally in models of somatic cell nuclear transfer

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(SCNT)¹⁶ or induced pluripotent stem cells (iPSCs).¹⁷ Both SCNT and iPSCs require donor cells, which are normally cultured primary cells from animals that exhibit a finite proliferative life span.¹⁸ Thus, SCNT and iPCS can reset the cellular aging clock in somatic cells.

As with cloning, iPSCs can generate an entire mouse embryo,¹⁹ demonstrating that nuclei of adult somatic cells can be rejuvenated and have their pluripotency restored. Although inducing pluripotency is different from increasing life span, these experiments reveal that the aging clock can be restarted, at least at the cellular level. As a consequence, species survive and diversify through the ages while stem cells navigate in the soma.

Vascular Aging: The Inflammatory Link

We can define aging as the set of processes that progressively reduce the time before an individual is likely to suffer a permanent loss of physical or mental capacity.²⁰ Although the extent of aging varies among individuals, no one escapes age-related pathologies like sarcopenia, cognitive dysfunction, atherosclerosis, osteoporosis, insulin resistance, cataracts, arthritis, hypertension, etc. But what causes this systemic decomposition? The hypothesis we present proposes that aging may begin in the vascular system, mainly in endothelial cells (ECs), which are linked to adult stem cell niches. Several lines of evidence indicate that vascular endothelial dysfunction develops with aging in humans in the absence of clinical cardiovascular disease (CVD) and major risk factors for CVD.^{21–23} Impaired endothelium-dependent dilation,²⁴ reduced fibrinolytic function,²⁵ increased leukocyte adhesion,²⁶ altered permeability, and/or other markers of endothelial dysfunction^{22,27–29} have been observed in older humans, as well as in rodents and non-human primates.

ECs are exposed to a range of stressors that may lead to endothelial injury. When ECs are activated by cytokines, oxidative stress, inflammation, and other signals, diverse protective mechanisms are induced that regulate genes involved in cell cycle, differentiation, senescence (*e.g.*, p53, p21, p16, p27), and survival pathways. Aged ECs display permanently activated routes, including an augmented pericellular proteolytic activity, a more disordered extracellular matrix, an increased inflammatory adhesion molecule expression, and abnormal cytoskeletal components.^{30,31} Abundant experimental and clinical data have demonstrated that aging is associated with chronic low-grade inflammation.³² Even in normal healthy aging, there is a pro-inflammatory shift in the expression profile of vascular genes, both in laboratory rodents and in primates.³³ In patients without cardiovascular risk factors, studies reveal increased plasma concentrations of several inflammatory markers (*e.g.*, tumor necrosis factor- α [TNF- α , soluble vascular cell adhesion molecule-1 [sVCAM-1], sE-selectin, interleukin [IL]-6, IL-18, and monocyte chemoattractant protein-1 [MCP-1]) that are positively related with age.²⁶ Therefore, these high levels of inflammatory cytokines and adhesion molecules create a pro-inflammatory microenvironment that results in vascular dysfunction and endothelial apoptosis during aging.

Numerous studies have shown that endothelial activation and pro-inflammatory gene expression in aging are triggered by increased nuclear factor- κ B (NF- κ B) activation.³⁴ It is noteworthy, that mitochondria-derived hydrogen peroxide

(H₂O₂) contributes to NF- κ B activation and a shift to pro-inflammatory gene expression. In addition, mitochondrial changes in endothelium have been related to aging.³⁵ Mitochondrial oxidative stress has an important role in vascular dysfunction, which is further exacerbated by an increased activity of oxidases (including nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidases).³⁵ Increased NF- κ B activation during aging is likely responsible for the increased expression of nitric oxide synthase and adhesion molecules that increase oxidative stress, promoting a decline of vascular function. Therefore, we postulate a pernicious spiral whereby oxidative stress activates NF- κ B, which induces oxidative stress and enhances the pathological change. This shift in the microenvironment facilitates the development of vascular dysfunction and endothelial apoptosis during aging.³⁶

A key signature of aging is the vascular rarefaction that affects systemic microvasculature in all organs.^{37–45} It is thought that increased apoptotic cell death and reduced endothelial turnover contribute to the age-related microvascular rarefaction. Age-related microvascular rarefaction contributes to a decline in blood flow, which reduces metabolic support and increases ischemic injury, especially in tissues with high metabolic activity like brain and heart.⁴⁶ In addition, aging reduces microvascular plasticity and the ability of the circulation to respond appropriately to changes in metabolic demand (Fig. 1).⁴⁷

Extrinsic Aging in Stem Cells

Other components of our microvascular theory of aging are adult stem cells. The age-related loss of microvascular plasticity and rarefaction has significance beyond metabolic support for organs because stem cell niches are co-ordinated with microvascular cells.^{48–52} One of the main features in human aging is the loss of adult stem cell homeostasis. Organs that are very dependent on adult stem cells show evident defects of the adult stem cell niches in aged mammals. Regenerative capacity loss suggests that tissue stem cells diminish in number with age. For instance, telomere dysfunction in *Terc*^{-/-} mice affect most tissues that depend on adult stem cells, such as the germ line, gut, skin, immune system, bone marrow, liver, and blood vessels. These tissues are characterized by decreased cell proliferation and/or increased apoptosis, showing characteristics of an aged phenotype related to the impairment of adult stem cell survival. Telomerase activity is essential for maintenance of telomere length and regenerative capacity in stem cells. Telomerase-deficient mice display limited viability.⁵³ Telomere repeats in these mice are lost at a variable rate of 2–7 kb per generation resulting in telomere exhaustion and increased end-to-end chromosome fusions. Although F4 *Terc*^{-/-} mice mimic the aging phenomena to some degree, they show reduced number and proliferation of adult stem cells in all organs, a trend not necessarily always represented in all aged organs like brain,⁵⁴ bone marrow,⁵⁵ skin,⁵⁶ and skeletal muscle.⁵⁷

Aging at the Vascular Niche

Stem cells in adult organs exist in specialized niches that control their differentiation and self-renewal. The niche microenvironment controls stem cell number, differentiation, and behavior. In the bone marrow, ECs regulate hematopoietic stem cell (HSC) differentiation and mobilization.⁵⁸ In

FIG. 1. Changes to the vasculature within a niche affects stem cell function. Bone marrow: The abundance of phenotypically defined hematopoietic stem cells (HSCs) increases with advancing age. In addition, aged bone marrow shows an increase in myeloid progenitors and a decrease in lymphoid lineage. Brain: Stem cell proliferation in aged brain is not only caused by a general decline in total precursor cell numbers but also by subtype-specific alterations in the proliferation rate. Growth factors synthesized by brain endothelial cells (B41) regulate neurogenesis and neural stem cell (NSC) proliferation. Aging does not only reduces the number of distinct precursor cell subpopulations but also specifically modulates their proliferative properties and inflammatory factors, and vascular dysfunction changes the niche microenvironment affecting neurogenesis negatively. Muscle: Quiescent satellite cells are associated with the myofiber and near microvasculature. Endothelial cells release soluble factors that promote myogenic cell growth. Aged muscle shows reduction of the intramuscular capillary number and an increase thickness of the capillary basement membrane. Microvascular rarefaction hampers satellite cell function facilitating sarcopenia.

the brain, blood vessels regulate neural stem cell (NSC) proliferation and differentiation.⁴⁹ In addition, skeletal muscular progenitor cells are dependent on microvasculature for muscular turnover.⁵¹ The connection between stem cells and the vasculature contributes to tissue repair and homeostasis. Therefore, changes in the vasculature at the niche level affects stem cell function.

Hematopoietic Niche

The aging changes observed in HSCs appear to correlate with a malfunction of the vascular niche. In fact, the abundance of phenotypically defined HSCs in mice paradoxically

increases with advancing age.^{59–63} Also, HSCs show a skewed maturation toward myeloid cell fates and away from lymphoid lineages.⁶⁴ Thus, although HSC function evidently deteriorates with age, the number of HSCs does not decline. HSCs can be transplanted serially into sequential recipients and show persistent function for more than 8 years, thus, exceeding the lifetime of the original donor.⁶⁵ Consequently cell autonomous, replicative HSC fatigue does not occur during periods of normal aging. Aged humans also present an increased number of HSCs⁶⁶ and show similar potential to repopulate irradiated bone marrow in non-obese diabetic (NOD)/severe combined immunodeficient (SCID)/interleukin-2 receptor chain-null (NSG) mice.⁵⁵

Therefore, a change in the niche can be, at least in part, responsible for the switch toward myeloid cell fates and reduction of lymphoid lineages. Interestingly, inflammatory cytokines expressed by aged endothelium have a role in the modulation of stem cell differentiation. For instance, expression of IL-6 by aged ECs expands the primitive progenitor population and shifts the differentiation toward a myeloid lineage, thus, blocking lymphoid differentiation.⁶⁷ Some of the cell autonomous defects in aged HSCs can be mediated by epigenetic changes.⁶⁸ These changes can be triggered by environmental inflammatory signals⁶⁹ due to defects at the niche.

Neurogenic Niche

The age-related decline in neurogenesis reflects a general decrease of proliferation in the aged brain,^{70,71} but it still is not clear whether this is caused by the failure of precursor cells *per se*^{71,72} or by the reduction in cell proliferation.⁵⁴ Dividing NSCs interact with blood vessels at places that lack pericytes to form vascular niches within the adult sub-ventricular zone (SVZ).⁴⁸ Various lines of evidence demonstrate the relevance of growth factors synthesized by brain endothelial cells (BECs) of the vascular niche in the regulation of neurogenesis and NSC proliferation.⁷³

These observations support our hypothesis that age-related alterations in the vascular microenvironment might contribute to this decreased neurogenesis in the aging brain.⁷⁴ Although the precise mechanisms remain to be discovered, inflammatory factors expressed in aged endothelium are in part responsible for changes in neurogenesis found in aged brains. A recent study has shown a marked increase in transforming growth factor- β 1 (TGF- β 1) production by ECs in the stem cell niche of middle-aged mice. The increased synthesis of TGF- β 1 by BECs in the stem cell niche causes stem cell dormancy and increased susceptibility to apoptosis. Moreover, pharmacological blockade of TGF- β 1 signaling restored the production of new neurons and their integration into the olfactory bulbs of irradiated elderly mice.⁷⁵ In addition, the chronic elevation of TGF- β 1 generates deposit of basement proteins and results in Alzheimer disease-like cerebrovascular amyloidosis and microvascular rarefaction and dysfunction.⁷⁶

Skeletal Muscle

Some quiescent satellite cells in the muscle are associated with the myofiber in their sub-laminal niche and are prepositioned near microvasculature.⁷⁷ During aging, decline of skeletal muscle mass and performance is a biological process named sarcopenia. On the basis of the key role of satellite cells in myofiber repair, a reduction in their number and myogenic properties may inhibit muscle maintenance and contribute to sarcopenia. Muscle stem cells, or satellite cells, demonstrate a reduced capacity to repair damaged muscle in aged mice. Interestingly, these defects in the function of satellite cells are due principally to alterations in the niche and can be reversed by restoration of a youthful extracellular environment in parabiosis experiments⁷⁸ and by transplantation in young recipients.⁷⁹

Muscle neoangiogenesis is associated with differentiating myogenic cells.⁷⁷ ECs release soluble factors that promote myogenic cell growth.⁷⁷ IGF-1, hepatocyte growth factor

(HGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor-BB (PDGF-BB), and vascular endothelial growth factor (VEGF) account for 90% of the EC-stimulated satellite cell growth.⁷⁷ Thus, myogenesis is accomplished through the secretion of soluble factors by ECs. Aged muscle shows reduction of the intramuscular capillary number and an increased thickness of the capillary basement membrane.⁸⁰ Consistently, all of these findings support that endothelial dysfunction affects satellite cell regenerative potential and participate in sarcopenia progression.

Germ Line Stem Cells

Another example of non-cell autonomous stem cell aging can be found in the ovaries. Ovaries are the organs that show the earliest impaired function in response to aging. This has been challenged by the formation of immature follicles in grafts of aged ovarian tissue into a young host ovary. Conversely, exposure of young tissue in an aged environment resulted in reduced number of immature follicles.⁸¹ Hence, failure of the stem cell niche rather than loss of oocytes is responsible for the aging ovary. It is also interesting that aneuploid conceptions increase with maternal age.⁸² Because oocytes are arrested in meiotic prophase, aging has to induce changes in the oocyte maturation. Follicular granulosa cells are a specialized endothelial-like cell population that nourishes the oocyte during maturation. Human and murine follicular granulosa cells express a set of phenotypic and functional markers that characterize them as specialized, endothelial-like cell populations.⁸³ Granulosa cells increase the number of apoptotic cells in older women.^{84,85} Moreover, pathologies like obesity⁸⁶ and diabetes,⁸⁷ which courses with vascular dysfunction, also increase granulosa cell apoptosis. Notably, VEGFR-2 activation levels are reduced in aged ovaries.⁸⁸ Although more data are needed to link ovarian aging and vascular rarefaction, indirect evidence suggests a relationship between oocyte maturation, aging, and vasculature.

Intrinsic Stem Cell Aging

During each cycle of DNA replication, telomere shortening, chromosome rearrangements, and single-base mutations lead to cellular senescence. Stem cells are positive for telomerase and exhibit longer telomeres,⁸⁹ and senescence-promoting pathways (p16INK4a, ARF, p53, FOXO, etc.) are repressed in true stem cell compartments.⁹⁰ Indeed, under homeostatic conditions, there is limited proliferative demand on self-renewing stem cells, sparing stem cells the perils of DNA replication and mitosis. Additionally, as stem cells become less metabolically active in their quiescent state, they are subjected to decreased DNA damage induced by metabolic side products such as reactive oxygen species (ROS).⁹¹ Therefore, if aging is influenced by ROS production, stem cells should display a lower aging ratio than adult cells. Some cells with high metabolic activity (like cortical neurons or cardiomyocytes) are not replaced during life,^{92,93} thus, there is no reason to think that a cell that is adapted to survive the "soma" should be the first that is affected by aging.

Several lines of evidence emphasize that epigenetic changes within adult stem cells in response to environmental cues are important to the regulation of stem cell aging. The best-characterized chromatin regulator of adult stem cells is

BMI1. BMI1 controls stem cells via the key “aging locus” p16INK4a/p19ARF.⁹⁴ In addition, factors associated with longevity have potential as chromatin modifiers, like sirTuins, FOXO,⁹⁵ and NF- κ B.⁹⁶

It is also important to remark that, although adult stem cells undergo changes with age, it is difficult to dissect which of these changes are causing intrinsic cell aging and which are consequences of the aged environment. Accumulation of toxic metabolites and oxidative stress can be caused by inadequate nourishment. Defective differentiation can also be caused by environmental changes. Some of these defects are able to endure, even after a transplantation in a young environment, but aged stem cells can also be preconditioned by changes acquired previously.

A Vascular Perspective on Aging

The vascular hypothesis of aging was initially predicted by the 17th century British physician Thomas Sydenham who stated: “A man is as old as his arteries.” This was interpreted to mean that arterial aging determines life expectancy based on CVD risk. However, recent data suggest that Sydenham’s comment may be interpreted beyond the pure implications for CVD. There is now sufficient experimental data to hypothesize that aging is, at least in part, the result of microvasculature decline that supports stem cell niche.

It is also interesting that some interventions of life extension like CR are powerful microvascular protectors.^{97,98} Other benefits of CR, such as the protection against insulin resistance/type 2 diabetes and hypertension, are also implicated in preserving microvascular function. Moreover, obesity is implicated in systemic inflammation, mediating vascular rarefaction, and dysfunction. In addition, vascular rarefaction in adipose tissue resulted in adipocyte dysfunction, causing an inflammatory milieu in the organism. Thus, CR preserves vascular function and reduces systemic inflammation, thereby promoting tissue nourishment and function. Even with no direct data, we can assume that some of the CR outcomes rejuvenating stem cell function, like muscle⁹⁹ and brain,¹⁰⁰ are mediated by its protective role in vascular function. Additionally, CR also delays aging in the reproductive¹⁰¹ and immune systems¹⁰² and restores circulating inflammatory cytokines to levels comparable with young animals.¹⁰³

Future Perspectives

Whether the microvasculature decline is the cause or the effect of other phenomena will undoubtedly be determined by ongoing research efforts. Three biological processes seem to play a role in organismal aging—microvascular rarefaction, adult stem cell dysfunction, and inflammation. They interact with each other to amplify a downward spiral that culminates in the death of an organism. Vascular rarefaction modulates stem cell niches, and this modulation, especially in bone marrow, generates a pro-inflammatory signature that negatively afflicts the endothelium.

In a sense, aging is a kind of disease—the definition of the disease state can be found in the aging phenomena. Thus, it is important to distinguish between two important research outcomes—one to prolong life that would treat symptoms and the other aimed at slowing the progression of aging progression that might lead to anti-aging therapies. Our

theory proposes a degenerative spiral where each force enhances the other two factors, promoting degenerative pathology. Age-related loss of plasticity and rarefaction of microvasculature in the stem cell niche is a potential powerful target for anti-aging therapy. Thus, focusing efforts and resources on this particular area could be very fruitful because multiple molecular and pharmaceutical tools might be used to dissect this phenomenon and translate results to the clinic. Interestingly, multiple molecules identified as aging palliatives, like anti-oxidants, also have a strong anti-inflammatory activity and work as protective agents for ECs. Moreover, regeneration aims to restore youthful properties to aged tissues for therapeutic purposes, for example, to improve immune system responses in wound healing or to improve cardiac function in the aged heart. The mechanism we have proposed for aging is based on the perspective of aging as a systemic disease and requires that we find solutions for this complex process. Human beings are by nature curious. Thus, we believe that inquisitiveness will drive the necessary research to solve many of the riddles associated with aging.

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Author Disclosure Statement

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