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Aging stem cells, latexin and longevity

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Stem Cells

The concept of the stem cell derives from the study of embryogenesis and has a strong historical basis in the field of developmental biology[1]. Adult stem cells comprise a small, highly regulated cell population characterized by their capacities for self-renewal and differentiation into a variety of mature cell types. Stem cells reside in a growing list of tissues and organs and participate to various extents in the replenishment of mature cells responsible for the specialized functional properties of the tissue in which they reside. It is because of these properties that stem cells are at the center of attention for the treatment of congenital and acquired diseases, and amelioration of the deleterious effects of aging. The degree to which the developmental potential of adult stem cells from a given tissue is restricted to the tissue in which it resides, is open to a debate that is difficult to resolve since stem cells circulate continuously in the blood and lymphatic vasculature[2]. The recent discovery that fully differentiated epithelial cells from adults can, through the (re-)initiation of expression of as few as four genes, appear to acquire most, if not all, of the attributes of embryonic stem cells with pluripotentiality, demonstrates the close affinity between at least some differentiated cells and the cells from which they ultimately arose[3,4]. It must be emphasized that these findings have been obtained under experimental conditions not encountered in nature; nonetheless, they may enable clinical tissue regeneration for the treatment of a number of diseases. If many adult tissues and organs are continuously replenished by cells derived from stem cells, then why do they show signs of aging? One possibility is that stem cells themselves age and senesce, resulting in a decreased ability to replace worn-out progeny and/or the fact that they pass on aged phenotypes to their progeny.

Missing in this discussion until now is the effect of the cellular and molecular environment on stem cell properties, although the molecular re-programming of epithelial cells into pluripotent stem cells demonstrates the importance of the intracellular environment. Indeed, ample evidence exists showing that intrinsic and extrinsic regulators are inextricably linked in determining stem cell functional properties. Of special current interest is the extracellular stem cell environment, commonly referred to as the stem cell 'niche', as originally coined for hematopoietic stem cells in the bone marrow. The cells and their extracellular matrices comprising the 'niche' for stem cells in different tissues are likely to be different, but the signals that mediate effects on stem cells, such as maintaining them in a replicative quiescent (or active)

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state often involve similar if not identical pathways (see review in ref. [5]). Moreover, the cellular and molecular composition of 'niches' is probably dynamic and responsive to needs for stem cell renewal and the differentiation of progeny into specific lineages.

Stem cell aging

In most tissues, aging has the effect of blunting processes akin to regeneration, such as wound healing[6] and hematopoietic reconstitution after chemotherapy or following bone marrow transplant[7,8]. Moreover, the role of stem cell aging in the extent and pace of regeneration remains to be fully explored. There is evidence in the hematopoietic system that stem cells progressively lose the breadth of their developmental potency. Serially transplanted bone marrow stem cells rapidly lose the capacity to produce the normal spectrum and proportions of blood cell lineages[9–13]. These studies suggest that stem cell plasticity, if such exists, may be higher when the organism is young and diminishes, or is lost, with age[14]. Highly purified young stem cells engraft the marrow of young recipients with high efficiency[15,16], but old stem cells have considerably decreased ability to home to the marrow and contribute to engraftment [17–19]. In addition, age-related changes in the functional abilities of HSC are clearly demonstrated by transplantation experiments using embryonic and adult cells, e.g., HSC from old bone marrow were capable of fewer repetitive transplantations than those from young marrow[20], HSC from fetal liver engraft better than adult mouse bone marrow cells in lethally irradiated mice[21], and limiting dilution repopulation assays show that fetal liver stem cells have extensive functional advantage over young adult bone marrow[22]. Thus, what are the changes seen in stem cells during the aging process?

A number of factors may interfere with the overall potency of the stem cell population during aging. However, much of the difficulty in assessing the effect of age on the stem cell population has to do with the way in which stem cell properties are measured. Functional tests require the proliferation and differentiation of stem cells, because it is their progeny that are actually measured. In transplantation experiments, engraftment by hematopoietic stem cell progeny is measured. In secondary transfers, it is stem cells produced by self-renewing stem cells of the primary graft that in turn generate progeny responsible for engraftment in secondary hosts. Under the best conditions, it is not possible to exceed five successful passages[23]. Failure to fully regenerate the stem cell population may be due to intrinsic effects such as aging and extrinsic effects associated with the transplant procedure[23–25]. Extrinsic factors associated with the transplant procedure, such as HSC reassociation with bone marrow stromal components and their integuments, may be responsible for the failure of the transplanted HSC to fully regenerate the host hematopoietic system[26].

Stem cells numbers

If stem cells play a role in the limitation of organismal longevity, the simplest explanation of how they might do so would be a decline in their numbers in old age. Qualitative changes in stem cells and in the composition of the stem cell population with respect to its qualities may be an important additional factor in stem cell aging. The amount of active bone marrow in humans progressively diminishes from childhood to old age, during which time active marrow is restricted to the pelvis, sternum, and vertebrae, and where even in these sites the marrow cellularity is reduced[27]. Moreover, the number of CD34+ cells in the marrow and blood of the very old, including centenarians, is greatly reduced[28,29]. Despite these quantitative changes there is little solid evidence that longevity is threatened by a lack of stem cells[30]. Rather, as discussed below, the response of old stem cell populations in many tissues is a lack of the robust response of their young counterparts to stress.

There is little question that cellular senescence is intimately involved in the aging process and we have championed the idea that the effects of age on the stem cell population contribute,

perhaps to a large extent, to the declining function of tissues during aging, and perhaps to organismal longevity[14,31,32]. Since the regulation of stem cells is accomplished via both intrinsic and extrinsic mechanisms, senescence, at both population and cellular levels, may involve age-related changes in either or both pathways. In this review, we examine cellular senescence from the vantage point of stem cells, particularly hematopoietic stem cells (HSC) in the bone marrow in the context of aging. Stem cell populations, by necessity, must persist for the lifetime of the organism, and for this reason, elaborate mechanisms must have evolved to preserve the cardinal stem cell functions. Nonetheless, there is strong evidence despite these protective mechanisms, that stem cell populations are not spared from the ravages of aging [32,33]. In this review, we propose that protein mis-folding and aggregation may contribute to stem cell aging in ways that parallel amyloidoses in the nervous system, such as Alzheimer's disease.

Senescence of a stem cell population can have quantitative and qualitative dimensions. For example, we have shown in embryo-aggregated chimeric mice that stem cell populations of the two composite strains [C57BL/6 (B6) and DBA/2 (D2)] contribute to hematopoiesis in completely different patterns during aging, despite their co-existence in a common environment, and presumably exposure to the same 'niche' environment[31]. In old chimeras, stem cells of the D2 genotype were either completely exhausted, senescent, or otherwise quiescent, since they gave way completely to blood cell production derived from stem cells of the partner strain (B6). We subsequently harvested marrow from chimeras in which the D2 stem cells were no longer contributing to hematopoiesis and showed that they were still present and could be re-activated by bone marrow transplantation, albeit with a greatly diminished duration of activity[34]. These results affirm the importance of intrinsic stem cell regulators and show that at least in this type of experiment, senescence at the stem cell level, if it occurs, must be reversible. Since cellular senescence has come to imply a series of irreversible changes, perhaps the more broad term, quiescence, better explains the changes observed during aging of D2 HSC. Much of the work on murine HSC has been carried out with B6 mice, often because a strain congenic for the pan-hematopoietic cell surface marker CD45.1, derived from the SJL donor strain, is readily available on the B6 genetic background. B6 mice carry the CD45.2 allele and thus in bone marrow transplant studies involving the two strains, donor and recipient hematopoietic cells can be easily distinguished and quantified by flow cytometric immunophenotyping. In contrast to diminishing HSC at old age in the relatively short-lived D2 and BALB/c strains, HSC numbers increase steadily in long-lived B6 mice up to an age of at least 30 months[35]. Whether the increased numbers of HSC during aging in B6 mice contributes to their longer lifespan is not known. What is known is that qualitative changes in B6 HSC, as in all other strains studied, accompany aging. For example, HSC from old mice have a biased differentiation pattern favoring myeloid differentiation at the expense of lymphoid lineages[18,36]. Therefore, it would be reasonable to propose that as stem cells have accumulated intracellular damage as a result of the rigors of aging, surveillance pathways leading to apoptosis, such as the p53-initiated cascade, takes care of compromised stem cells by removing them from the pool, preventing the establishment of a clone with potentially dysfunctional or tumorigenic progeny[37–39]. The importance of apoptosis in the physiological regulation of HSC population size suggest that this mechanism may be a target of aging. For example, overexpression of *bcl-2* in transgenic mice not only prevents apoptosis in response to a number of genotoxic challenges, but increases by more than twofold the number of HSC in these mice under steady-state conditions and enhances their engraftment potential on a cell-by-cell basis[40]. If cells are lost through accumulated damage, could stem cell replication recover stem cell numbers?

Stem cell replication and the effect on telomere length

A hallmark of the population of stem cells from normal young and middle-aged mice or humans is its overwhelming quiescence[41,42]. Bromodeoxyuridine (BrdU) administration has been used to label the stem cell compartments of young, but not old, mice. In young marrow, as expected, a short pulse of BrdU has a very small labeling index, consistent with a quiescent population. Long-term BrdU administration to young mice via drinking water showed that essentially all stem cells replicated at least once about every 2 months[43,44]. These results are in disagreement with the concept that most stem cells are deeply quiescent, and many of which may not enter cycle during a mammal's entire lifetime[45]. The pattern of cell cycle kinetics of stem cells may be altered during aging[35], but further studies are required in both mouse and man to resolve this issue. However, an increased proportion of stem cells in cycle might be correlated with the increased incidences of leukemia, lymphoma, and myelodysplastic syndrome at old age[35].

Interest in the importance of telomeres to mammalian aging, replicative stress, and cancer etiology in the last several years has led to the their study in stem cells[46–52]. The assumption has been that telomeres serve as a pacemaker of senescence in replicating stem cells, as in other cell types, in which the length of telomeres shortens with each round of replication until a critical short length is reached that signals induction of cell senescence[53,54]. Despite the fact that HSC produce telomerase[55–57] telomeres of stem cells have been shown to progressively shorten during aging[49,58–60] and following hematopoietic stress[61], especially that following stem cell transplantation[59,62–66]. Recent studies have shed light on the question of whether telomere length in stem cells plays a role in their function under physiological conditions. Inter-species comparisons of stem cell numbers in mice, cats and humans revealed that, despite the variation in sizes and lifespans of the three species, the total number of hematopoietic stem cells did not reflect those variations and were, in fact, remarkably similar [67]. By measuring telomere lengths in blood granulocytes, and applying stochastic simulation, Shepherd et al.[68] and Lopes et al. [69]have shown that while the absolute number of stem cells may not reflect the variation in organismal size or longevity, the rate of hematopoietic stem cell turnover does. Replication rates for HSCs in mice, cats and humans were estimated to be once in 2.5, 10, and 45 weeks, respectively[68]. Thus, the way in which stem cell populations are organized and parsed for use is a reflection of a combination of physiological parameters including the time-scale over which functional activity is required.

Organismal Aging

If aging is viewed as the cumulative wear-and-tear on cells of an organism, the rate and degree of organismal aging is a reflection of how well the organism counteracts the deleterious effects of the environment. Currently, two major theories regarding organismal aging are proposed: an evolutionary-based theory and a damage-based theory. Many evolutionary biologists suggest that genes that favor the species' reproductive success may have negative effects in later life, thus limiting the organism's life span. This phenomenon has been termed antagonistic pleiotropy[70]. According to this theory, organisms are effectively maintained only to achieve reproductive success. Following a species' reproductive phase, genes selected for their beneficial effects were not selected in later life. An example of antagonistic pleiotropy is the role of androgens in males. Androgens, the hormones responsible for the normal growth and function of the prostate in the young, and pivotal in the generation of sperm for reproduction, are the same hormones that contribute to prostate cancer in the elderly.

The replicative senescence (replicative damage) theory was first introduced in the 1960s as a process that limits the number of cell divisions that a specific cell can undergo throughout life [71], and has subsequently been linked to the loss of telomeric DNA described above.

Therefore, replicative senescence, or growth arrest, was postulated to be a direct link to how many rounds of replication that cells undergo during their lifetime[72]. During each cell division DNA is lost at the ends of the chromosomes[73] because most of our somatic cells do not express telomerase, the enzyme responsible in reconstituting the structures at the ends of the chromosomes, the telomeres[74]. Joeng et al. [75] have shown that life span could be prolonged in worms that overexpressed HRP-1, a telomere binding protein that steadily increases telomere length. However, the mechanisms involved in such a process are unknown. Further studies have shown that the affected cells underwent senescence before they acquired critical short telomeres, avoiding the genomic instability leading to cancer. Therefore, replicative senescence/growth arrest did not depend exclusively on telomere erosion – it depended on other mechanisms as well.

DNA replication itself imposes stress in the proofreading and editing mechanism necessary to keep the genome free of replication errors. The DNA repair machinery plays a pivotal role in keeping the genome free from mistakes that invariably occur during replication and that are caused by environmental insults. Therefore, there appears to be a direct link between life span and DNA repair[76]. Species that have efficient DNA repair machinery live longer than those without this capacity[77]. Mutations in DNA polymerases have also been implicated in the aging process and, not surprisingly, senescent cells have faulty DNA polymerases[78].

Throughout life, molecular mechanisms necessary to counteract the deleterious effects of the environment evolve[79]. In addition to cellular senescence, apoptosis is an important evolutionarily conserved mechanism used to remove damaged cells. Unlike necrosis, it consists of the removal of unwanted and damaged cells without exposing neighboring cells to cellular proteases. Defects in the regulation of apoptosis have been linked to degenerative and hyperproliferative diseases such as cancer[80–82]. Key molecules in this process, such as p53, Bmi-1, p16ink4a, p19Arf, and bcl-2 have been identified and strongly linked to apoptosis and senescence[83–88]. Therefore, as in senescence, apoptosis is a tumor suppressor mechanism that prevents cells carrying mutations to divide and generate defective progeny. Apoptosis and senescence may have evolved together to protect complex organisms that have a mix of proliferative and postmitotic tissues.

Aging Damage

A theory based on cellular damage was first proposed by Harman in the mid-1950's[89]. Today it has two main components: oxidative damage and cellular/replicative damage (Figure 1). Normal metabolism produces reactive oxygen species (ROS) that may oxidize and damage cell membranes, proteins, and nucleic acids. An example of the effect on the aging process of ameliorating ROS toxicity may be found in studies in which enzymes that limit exposure of cellular components to ROS, such as catalase and superoxide dismutase (SOD1), were over-expressed. Supra-normal levels of SOD1 in *Drosophila* caused an increase of 20–30% in life span compared to control flies[90]. Interestingly, if over-expression was targeted to neuronal cells, flies lived even longer than flies that have total body overexpression of the enzyme, thus confirming that the nervous system is susceptible to damage by ROS and that such damage affects longevity. In mammals, the link between ROS and aging is still incomplete because there is no evidence of premature aging in mice carrying loss-of-function mutations in ROS degrading enzymes[91]. In addition to ROS, damage in the mitochondrial genome has been closely linked to aging. Mutations in mitochondrial DNA cause defects in the electron transport chain that affects energy production and creates ROS. Age-dependent declines in mitochondrial function are present in many species[92], supporting a current view that aging may be directly related to cellular metabolism and decreased mitochondrial function[93],

In addition to mitochondria, other intracellular organelles such as lysosomes are damaged over time due to free radical-mediated modifications[94]. Lipofuscin, the age pigment first described in brain cells of the elderly, is composed of intra-lysosomal polymeric material that cannot be degraded by lysosomal hydrolases[95]. There is an inverse correlation between lipofuscin accumulation and lysosomal function, and consequently cellular life span[96]. In short-lived species, mitochondria release more electrons and peroxides than long lived species and therefore accumulate more lipofuscin granules[97]. The presence of lipofuscin in stem cells is still unknown. Only recently has the accumulation of lipofuscin been shown to interfere with cellular functions and promote age-related pathologies such as neurodegenerative diseases, heart failure, and macular degeneration[98].

Aging and protein mis-folding

Protein mis-folding is a common event in living cells, that possess a constant risk over the life of the individual[99–102]. In young and healthy cells, misfolded proteins are efficiently eliminated by an intracellular defense system[103]. The system involves production and maintenance of functional proteins, precisely regulated turnover, and the removal of damaged and aberrant proteins[104,105]. In aging cells and in cells from individuals with specific genetic diseases, the misfolded protein load may overwhelm the system and disturb its protective function, leading to abnormal protein accumulation and subsequent self-assembly into toxic oligomers and aggregates[106,107]. Many mechanisms may be involved in the increased risk of protein mis-folding and aggregation in senescent cells. Oxidative stress and ROS represent the major contributors[108–110]. Accumulation of ROS during biological aging ultimately leads to the widespread oxidation of biomolecules, including DNA, lipids and proteins. Oxidatively modified proteins are thermodynamically unstable and prone to structural alteration, thus promoting protein self-assembly and aggregation[111–113]. A second mechanism involves accumulation of mutated DNA with age due to increases in DNA damage and a decline in DNA repair processes[114,115]. When non-synonymous mutations occur in coding sequences, inappropriate amino acids may be substituted, which may destabilize the native folded structure of proteins and favor the formation of mis-folded proteins[116]. When mutations occur in promoter sites of age and disease-associated genes, they may increase transcription and subsequently the concentration of aggregation-prone proteins and peptides [117–119]. The misfolded proteins and their aggregates may interfere with the normal cellular functions in a multitude of ways, all of which ultimately lead to senescence or cell death. As discussed previously, oxidative stress, DNA mutation, and perhaps other mechanisms may be involved in age-related declines in stem cell pool size and functions. Although very little is known about the role of misfolded and aggregated proteins in senescent and cancerous stem cells, it potentially represents a major determinant in defining the stem cell functions, the rate of aging, the development of age-related diseases and, yes, lifespan.

Latexin and cystatin C

The qualitative changes in stem cells and the composition of the stem cell population with respect to qualitatively distinct subclasses is an important factor in stem cell aging. We have shown that amongst mouse strains there is a strong correlation between the rate of early hematopoietic progenitor proliferation and mouse lifespan[120,121]. Moreover, we and others have observed large strain-specific differences in the maintenance of the HSC population during aging[36,120,122–126], thus suggesting that genetic regulation plays an important role in the way aging affects HSCs. Using forward genetics, we recently identified a protein, latexin, whose differential expression in stem cells accounts for at least part of these differences in young murine hematopoiesis[127]. We have showed that latexin is a negative regulator of stem cell number and acts through at least two mechanisms to modulate stem cell pool size: a) it decreases HSC cell replication and b) it increases HSC apoptosis. Therefore, in the

hematopoietic system, and perhaps other organs, latexin influences aging and perhaps lifespan through its action on stem cells.

Latexin was originally discovered in the lateral neocortex of rats and acts as a marker of regionality and development in both central and peripheral nervous system[128,129]. It was also expressed in a number of other tissues, including hematopoietic and lymphoid organs [130]. It is 222 amino acids in length with a molecular weight of 29kD. Latexin is the only known carboxypeptidase inhibitor (CPI) in mammals. It is a non-competitive, nearly irreversible, and potent inhibitor of carboxypeptidase A (CPA), but is less potent against carboxypeptidase B (CPB) and does not act on various other proteases[131–133]. In rodents, latexin inhibits CPA1, 2 and mast cell CPA (CPA3), whereas it binds to CPA4 in humans[134]. Latexin may thus function in regulating tissue-specific protein degradation and turnover. As an endogenous carboxypeptidase inhibitor, the latexin primary sequence, however, doesn't possess significant homology with other reported CPIs[135]. Instead, it shares high similarity in structure with a cysteine protease inhibitor, cystatin C. Latexin consists of two topologically equivalent subdomains, each with a cystatin-like topology[132]. Latexin and cystatin C have structural and perhaps evolutionary ancestry, in common, but they also have their own specific characteristics as summarized in Table 1. First, functional domains and/or conformation may be different between cystatin C and latexin because they have different targets. Second, latexin is localized in the cytoplasm due to the lack of a membrane-specific signal peptide sequence, whereas most of cystatin C is secreted into biological fluids. Therefore, the inhibition of cystatin C on cysteine protease takes place extracellularly, whereas latexin mainly regulates cytosolic proteins. Interestingly, latexin does not interact with its CPA target under normal conditions, because studies have shown that they are not co-localized in the same granular compartment in the cytoplasm, at least in rat peritoneal mast cells[136]. These results suggest that either latexin does not function through its inhibition of CPA, or the inhibition occurs under certain conditions when CPA is released accidentally into the cytoplasmic space and latexin granules intercept the released CPA and inhibit it. Third, it has recently been shown that latexin and cystatin C can be induced to form amyloid- β like aggregates, although through different self-assembly mechanisms and under different induction conditions. A recent study has revealed that *in vitro* assembly of latexin is initiated by its conformational change under conditions where the polypeptide chain is mainly unfolded[137]. In contrast, polymerization of cystatin C takes place under conditions where the polypeptide sequence is altered because of genetic polymorphisms or mutations[138]. Cystatin C variants or mutants are less stable and prone to form the folded dimer intermediates, which are the building blocks for cystatin C oligomerization. Finally, cystatin C has a broad range of biological roles because it is secreted into bodily fluids and tissues[139,140], whereas latexin may function in specific cells depending on where it is expressed. Irrespective of venue, it is also possible they function in a similar manner due to their structural similarity.

Roles of latexin in stem cell aging

Despite several structure-function studies of latexin, there is still very little knowledge about its biological roles in stem cells and aging. We herein propose some potential regulatory mechanisms of latexin in stem cells, aging and age-related diseases. We have shown compelling evidence of latexin's involvement in the regulation of HSC in young mice. Our unpublished preliminary results revealed that latexin expression in fetal hematopoiesis and early adulthood are strongly correlated with HSC numbers throughout adulthood and old age. Although latexin was originally detected in differentiated neurons, some evidence indicates it is also present in neural stem cells (NSCs)[141,142]. According to the concept of adult stem cell plasticity and the noteworthy genetic overlap between NSCs and HSCs, our results showing regulatory roles in HSCs may also be applicable to NSCs. This hypothesis could be supported by a recent report showing cystatin C, a latexin homologue, regulated neurosphere generation

either by direct induction of embryonic stem cells (ESCs) into functional NSCs, or by expansion of cells that had spontaneously differentiated into NSCs[143]. These results underscore that in the hematopoietic system, and perhaps in other organs, latexin influences aging and perhaps lifespan through its action on stem cells.

Latexin and neurological amyloidoses

The deposition and aggregation of misfolded proteins is strongly implicated in the pathogenesis of aging-associated neurologic disorders, such as Alzheimer's disease (AD) and Parkinson's syndrome, to name just two[144]. Latexin was found to be significantly down-regulated in an AD mouse model, and the recent findings that cystatin C over-expression ameliorates this disease suggests that latexin may play a similar role[145]. Amyloid- β ($A\beta$) is the major constituent of the amyloid fibrils deposited in the brains of patients with AD. It is a processing product of a larger β amyloid precursor protein (β APP)[146]. Immunohistochemical studies have revealed the colocalization of cystatin C with $A\beta$ [147], suggesting that as a component, cystatin C either enhances amyloid fibril leading to neuronal degeneration, or inhibits fibril formation. Using an Alzheimer's disease mouse model, two studies from Levy and Jucker laboratories have shown that overexpression of cystatin C could diminish amyloid- β deposition and thus plays a protective role in AD[148,149]. The underlying mechanisms involve the direct binding of Cystatin C and amyloid- β rather than the effects on APP and amyloid- β expression level. The binding affinity between amyloid- β and cystatin C is high enough to prevent amyloid- β accumulation and reduce cystatin C self-association. Because of the structural similarity between latexin and cystatin C, latexin may act as another potential protective factor that prevents the development of aging-related neurological diseases.

Latexin and cancer

Increased propensity to cancer is one of prominent hallmarks of aging. Latexin shares 30% sequence similarity with Tazarotene-Induced Gene 1 (TIG1), which is down-regulated or absent in an extensive list of tumor types[150]. Studies by Callahan et al. revealed that the absence of a candidate gene, although yet unidentified, whose protein product had 85% identity of the mouse latexin protein, was associated with increased incidence of ovarian cancer[151]. We have found similar expression patterns of latexin in a variety of human leukemia and lymphoma cell lines, and in primary cells from patients with these diseases (manuscript in preparation), indicating that latexin may be implicated in the regulation of tumor progression, perhaps as a suppressor. Cystatin C has demonstrated both tumor-suppressing and tumor-promoting functions. It attenuated tumor cell-mediated invasion and degradation of extracellular matrix[152].

Overexpression of cystatin C in the mouse model of glioblastoma reduced intracerebral tumor formation[153], thus demonstrating anti-tumor effects. In contrast, transplantation of a highly metastatic melanoma cell line into cystatin C-null mice significantly reduced lung translocation of malignant cells, indicating that cystatin C may enable the metastasis of malignant cells [154]. The dual role cystatin C in tumor progression could be explained by a combination of its protease inhibitor activity and cytokine-like activity[155]. As a member of the cystatin superfamily, latexin may act similarly in the malignant transformation of stem and progenitor cells and regulate tumor progression.

Many mechanisms are involved in the aging and age-related decline in tissue functions. Stem cells and their changes with age are of great interest because they contribute to life-long replenishment of functional mature cells. Genes implicated in the stem cell regulation would provide a good platform for studying the relationship among stem cells, aging and age-related diseases. Using latexin as an example, we put forth a hypothesis addressing the questions of how genetic changes affect stem cells, the nature of underlying mechanisms and how the aging

phenotypes are interconnected with each other (Figure 2). Latexin initiates cellular responses through three potentially related mechanisms: 1) inhibition of carboxypeptidase A, 2) participation of intracellular signaling pathways, and 3) regulation of protein aggregation. These processes, perhaps along with other mechanisms, synergistically or independently regulate cell proliferation and apoptosis, that consequently affect stem cell numbers and function. When latexin expression increases in stem cells with age, as we have observed in human hematopoietic stem cells, the stem cell pool size will become smaller and tissue regeneration capacity in the face of stress may be impaired, a process fostering stem cell aging. On the other hand, high levels of latexin may inhibit protein aggregate-induced cellular toxicity in old stem cells and prevent their functional decline with age. As a putative tumor suppressor, elevated latexin level could reduce the increased propensity of senescent stem cells to transform into cancer stem cells and in result in tumor formation. Thus, latexin may exert an anti-aging effect at the intersection of stem cells, aging, and cancer in ways that come full circle with events in embryogenesis and tissue regeneration.

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Figure 1. Biological processes affecting stem cells

The biology of stem cells involves many different cellular processes that take place in order to maintain the appropriate number of HSCs and their functionality during variable environmental conditions throughout life. The stem cell pool is carefully maintained through a balance among proliferation vs quiescence, self-renewal vs differentiation, and the dynamic interplay between extravasation into the blood and lymph (mobilization) vs lodement in a bone marrow niche (homing). Any defect in any of the processes will apply stress on HSCs and lead them to senescence and apoptosis. The compromise of stem cell pool size and their functions will ultimately result in early “aging” involving loss of their normal potential and thus affect longevity. The green arrow indicates cellular processes favoring maintenance of HSC population, whereas brown arrows indicate those factors compromising HSCs. HSCs: hematopoietic stem cells. HPCs: hematopoietic progenitor cells.

Figure 2. Potential roles of latexin in stem cell senescence

Three potential mechanisms may be involved in the regulation of stem cells by latexin: inhibition of carboxypeptidase A (CPA), involvement of intracellular signaling pathways and protein folding. In young stem cells, these mechanisms are well regulated and the biological processes affecting stem cells are balanced. In sum, these result in the homeostasis and normal functionality of stem cell population. As latexin expression increases with age, latexin-associated biological processes are affected, and may contribute to stem cell senescence, and quantitative loss. Age-associated diseases, such as amyloidoses and cancers may also be related to latexin levels in old stem cells. Therefore, latexin represents a good model to address the relationship among stem cell, the rate of aging, the development of age-related diseases, and lifespan.

Table 1

Comparison of latexin and cystatin C.

	Latexin	Cystatin C
Peptide length	222 residues	111 residues
Cystatin-like domain(s)	Two	One
Cellular location	Cytosolic	Secreted
Building blocks for aggregation	Unfolded polypeptide	Partially folded polypeptide
Aggregation condition	In vitro Mild denaturing condition (3–8M urea)	In vivo Genetic mutation-induced amino acid substitution
Inhibition target	Carboxypeptidase A	Cystein proteases
Known functions	1) Regulation of hematopoietic stem cell pool size 2) Inflammation 3) Sensory perception 4) Neuron marker	1) Regulation of embryonic and neural stem cells 2) Immune response 3) Tumor progression 4) Alzheimer's disease 5) Bone formation 6) Renal function marker 7) Other functions

Note: references are cited as in the main text.