Stem Cells and Aging

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Introduction

Aging is the most important risk factor for various human pathologies, yet it is quite challenging to study owing to its multidimensional, multifactorial, and pervasive nature, affecting the entire organ systems of the body. The process of aging is driven by progressive loss of physiological, biochemical, and molecular integrity, leading to various diseases such as cardio-cerebro vascular and metabolic disorders, neurodegenerative diseases, diabetes, cancer, and eventual death [1]. The underlying causes include accumulation of subtle, irreversible cellular and molecular changes over an individual's lifespan, leading to progressive decline in the intrinsic regenerative and homeostatic potential. Such degenerative changes coupled with homeostatic alteration lead to stem cell exhaustion, genetic instability, cellular senescence, altered cellular communication, mitochondrial dysfunction, telomere attrition, multiple epigenome and transcriptome changes, loss of proteostasis, and deregulated nutrient sensing, among others [2]. However, the most important attributable factors among them are age-dependent changes and decline in tissue-/organ-specific stem cells. Such decline in stem cell population either precedes or followed by deterioration in niches surrounding them along with functional molecular cues that regulate their various biological activities. Repertoire of stem cells present in various organs steadily decline due to replicative aging that is preceded by global metabolic deterioration resulting from patho-physiological conditions which is, in turn, driven by

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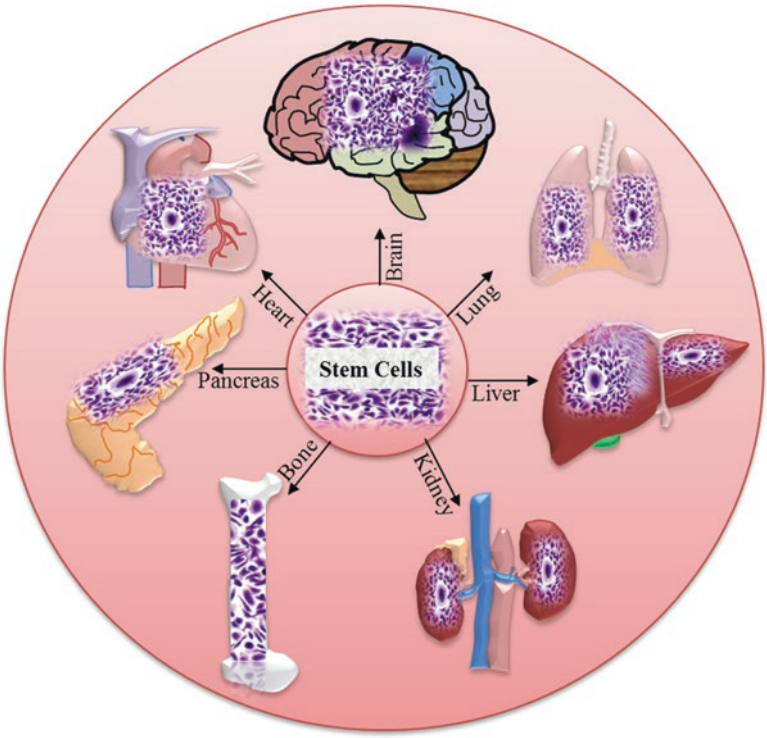

Fig. 12.1 Major locations of stem cells. Stem cells are located in almost every organs, including the brain, heart, lungs, liver, pancreas, major bones, and kidney, and help them remain functionally integrated and working throughout an individual's life. However, their associated functioning deteriorates as a function of aging, leading to physiological decline and onset of age-associated pathologies

aging process. Stem cells serve as cellular backup of various organs, continuously renewing them by supplying new daughter cells and requisite set of cytokines and growth factors required for regeneration and repair of tissues/organs so as to keep them integrated and functional throughout life span (Fig. 12.1). However, with the passage of time, stem cells also start showing multiple signs of replicative senescence and metabolic slowdown, thereby compromising on self-renewal and proliferation, cutting down the supply of new cells/secretory molecules and growth factors to the organs. These age-associated progressively deteriorating changes deprive the organ-specific niches/microenvironment of functional growth and molecules needed for mutually concerted functioning between stem cells and their respective organs [3]. The steady loss in the regenerative potential during aging is primarily attributed to telomere shortening/attrition, which flirts with the commitment of stem and non-stem cells [4], decrease in the ratio of DNA repair to damage, low level of ATP production owing to reactive oxygen species-induced (ROS) mitochondrial DNA damage, accumulation of damaged proteins and dysfunctional

organelles, epigenetic and epitranscriptomic modifications, and consequent deregulated gene and protein expression, among others. The aforementioned biological changes affect stem cells as well as non-stem cell population on several accounts, which together contributes to development of age-related pathologies, and hence drive organismal aging. Such scientific correlation between stem cells and aging has been quite significant in developing our understanding to a certain extent, and hence becomes even more important to look at the multidimensional and multifactorial aging process from the stem cells perspective, which will be helpful in improving health span of organisms, including human beings. Considering all the findings in an integrated and holistic way would certainly help the ever-expanding aging society and address the issues regarding increase in age-related psychosomatic health problems confronting the human society worldwide.

Hallmarks of Stem Cell and Organismal Aging

Aging is associated with subtle but steady decline in organs' functions and structure at both microscopic and macroscopic levels, and hence substantially increases the risk factors for developing age-associated illness and diseases. The major underlying causes for such deterioration include stem cell exhaustion, genetic instability, cellular senescence, altered intercellular communication, telomere attrition, epigenetic and transcriptomic changes, mitochondrial dysfunction, immunosenescence, and loss of proteostasis (Fig. 12.2).

Stem Cell Pool, Self-Renewal, Quiescence, Terminal Differentiation, and Aging

Stem cells are very dynamic in nature, which helps them fulfill the growth, maintenance and regeneration demands of aging tissues undergoing slow but steady molecular, functional, and structural changes over an individual's lifetime. For instance, they divide very fast during fetal development so as to keep pace with tissue growth and development within evolutionary-allowed developmental timeframe. But this rapid cellular proliferation slows down considerably by stage of young adulthood, and later on variably ceases in mature mammalian tissues as they undergo quiescence, with intermittent division to maintain tissue homeostasis. In old adults, stem cells show enhanced tumor suppressor expression, possibly to avert tumorogenesis at the expense of tissue's intrinsic regeneration potential. With the advance in age, tissue repertoire of stem cells starts declining due to intrinsic and extrinsic factor-induced cellular exhaustion. Stem cell exhaustion is usually found to be age-dependent that is induced either by slow decline in self-renewal ability with age or progressive changes in the niches surrounding the pool of functional stem cells in various tissues. There have been various comprehensive studies showing age-dependent perturbed cell cycle regulation and depletion in stem cell abundance in a range of tissues, including muscles, brain, germline, liver, bone marrow,

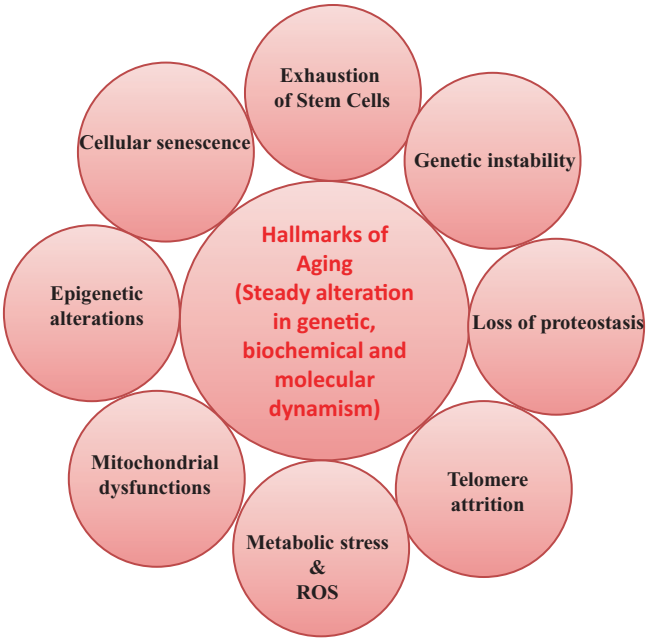

Fig. 12.2 Major hallmarks of aging. The diagram shows major hallmarks of aging, including exhaustion of stem cells, cellular senescence, genetic instability, epigenetic alterations, loss of proteostasis, mitochondrial dysfunctions, telomere attrition, metabolic stress, and reactive oxygen species (ROS)-induced changes

adipose tissues, etc. For instance, aged human brains have been found to possess significantly lower number, yet functional, of neuronal progenitor cells compared to young brains [5]. In addition, aged humans have lesser neurogenesis, but higher gliogenesis as opposed to their younger counterparts. However, there are few exceptions to the age-dependent decline of stem cells. For example, hematopoietic stem cell (HSC) populations have actually been found to increase in both number and frequency in aged mice, albeit, with reduced cell division and cell cycle progression, and higher accumulation of damaged cell cycle regulators such as p^{18} and p^{21} [6]. Furthermore, there has been empirical evidence suggesting subtle decrease in functionality of HSCs with each round of cell division [7], which may be further compounded by aging-associated DNA defects and resultant chromosomal lesions [8]. Apart from age-dependent decline in self-renewal ability, other underlying mechanisms may involve terminal differentiation, apoptosis, quiescence, differential niche-based selection pressure, and senescence of stem cells [9]. HSCs show age-dependent quiescence which, on the one hand, protects it from functional exhaustion and accumulation of damaged DNA and, on the other, promotes persistence of mutations as it allows the survival of cells with defective DNA. Surprisingly, cell cycle entry of damaged HSCs has been found to either help in DNA repair or getting the body rid of damaged and nonfunctional HSCs [10, 11]. Therefore, a

precise balance between cell division and quiescence is of utmost importance for the proper functioning and maintenance of hematopoietic tissues in order to support hematopoiesis. Hematopoiesis depends on multiple intrinsic and extrinsic regulatory factors which, in turn, are being prevailed over by various pathophysiological parameters, including stress, immunity, and aging. For instance, under homeostatic conditions, hematopoiesis is usually maintained by short-term HSCs, also known as early hematopoietic progenitor cells (HPCs), while the same switches to long-term HSCs in response to stress [12]. Similarly, aged niche imposes differential selection pressure on various types of HPCs, favoring the monoclonality at the expense of natural polyclonality, which might be one of the underlying causes for higher incidence of age-associated blood-related diseases such as leukemia [13]. Besides, hematopoietic stem or progenitor cells (HSPCs) undergo age-dependent genetic alterations, including base-pair mutation, deletion, duplication, and other potentially harmful chromosomal anomalies. Aging-induced accumulation of unresolved DNA damage triggers the cell-intrinsic aged phenotype. For instance, humans under the age of 50 years show low frequency (0.2%–0.5%) of such chromosomal lesion, which drastically increases up to 2%–2.5% by the age of 80 years. Therefore, individuals over the age of 70–80 years have relatively higher risk of developing hematopoietic cancer [14]. In general, with advance in age, HSCs acquire lymphoid to myeloid lineage bias, reduced regenerative potential, and a dominant expansion of myeloid clones toward malignancies (Fig. 12.3).

Aging tends to variably destabilize the genomic integrity of almost all types of somatic and stem/progenitor cells irrespective of their location and functional specialization. Several comprehensive studies have shed light on how the parental age affects the offsprings, and their likelihood of developing genetic diseases quite early in the life. Spermatogonial stem cells or germline stem cells in aged male tend to have multiple molecular and genetic alterations which confer partisan advantage to mutant cells over their nonmutant counterparts. For example, mutations in Ras pathway cause one of parental age effect (PAE) diseases in the offspring. In *Drosophila*, there is age-dependent increase in frequency of stem cells with misaligned centrosome, contrary to centrosome orientation checkpoint, preventing such cells from division, and hence consequent decrease in sperm production. This leads to "selfish" proliferation and exponential increase of mutant spermatogonial cells over neutral mutation-carrying spermatogonial stem cells in the testes of aging men [15], which may have huge impact on the genetic make of the offspring and fertility.

Aging, slowly but steadily, tilts the balance between growth and atrophy in skeletal muscles that is empirically attributed to age-dependent loss of skeletal/muscle stem cells (SMSCs). Both young and adult maintain differential proportion of muscle stem cells with elder having less proportion owing to prevalence of lower asymmetric division. During muscle growth and development, the muscle precursor cells fuse with pre-formed muscle fibers, resulting into generation of new muscles and correspondingly increased muscle mass. Post-natal muscle development is followed by slow myonuclear turnover at around 15 years during adulthood. Human myoblast quiescence results from age-induced methylation-based alteration in sprouty 1 pathway, impairing the self-renewal potential of aged muscle stem cells. Moreover,

Fig. 12.3 Age-related changes in various stem cell populations. Aging has adverse effects on various functions of stem cells such as self-renewal, proliferation and differentiation

cellular senescence coupled with apoptosis has been proposed to be underlying mechanisms for age-based muscle stem cell loss as evidenced by higher susceptibility of aged murine muscle stem cells to in vitro apoptosis. Moreover, aged muscle stem cells tend to show considerably higher expression of myogenic differentiation markers such as Pitx2, MYH3, and MYL1, on the one hand, while having down-regulation of sprouty1 and Pax7, markers of quiescent fate, on the other. Elderly muscle stem cells also possess inability to return to quiescence due to DNA methylation-induced suppression of quiescence pathways. Therefore, decreased asymmetric division/self-renewal in conjunction with higher likelihood of terminal myogenic differentiation may result into loss of reserved muscle stem cell pool in elderly and aged individuals [16]. Besides, there is an age-dependent changes in muscle stem cell niche which cause decline in their self-renewing ability due to excess proliferation in subset of satellite cells. For example, aged muscle stem cell niche, consisting of muscle fibers, expresses Fgf2, making subset of satellite cells, break quiescence, undergo proliferation-led depletion, and hence loss in long-term regeneration potential, whereas relatively dormant satellite cells have robust expression of Spry1, and that is why they do not undergo depletion, would persist even in

aged muscle, and could be attributed to little regeneration occuring in aged individuals. Therefore, inhibition of Fgf2-mediated signaling by overexpressing *Spry1* has been found helpful in preserving satellite stem cell pool and stem cell functions in aged muscle [17].

Metabolic Stress, ROS Generation, Oxygen Sensitivity, and Mitochondrial Dysfunction

Long-lived tissues with minimal turnover are quite susceptible to the accumulation of oxidative, especially reactive oxygen-induced and nonoxidative damages, triggering a series of radical change in cellular phenomena, including cellular senescence, cell cycle arrest, decreased tissue-damage repair and regeneration, and eventual cell death. The damaging ROS is profoundly generated as a result of electron "leakage" during universally occurring biological process, oxidative phosphorylation, in mitochondria. The ROS-induced molecular damages can be understood in light of "free radical theory of aging" postulated by Harman in 1972 [18]. As per this theory, age-based accumulated cellular damage and compromised mitochondrial integrity cause elevated ROS production, thereby further damaging cellular macromolecules and already compromised mitochondrial oxidative phosphorylation, leading to eventual cellular decomposition and cell death. Reactive oxygen species such as superoxide (O_2^-) and hydroxyl radical ($^{\bullet}OH$) are highly reactive and consequently short-lived, damaging cellular DNA, proteins, and lipids either by direct or indirect chemical addition and/or modifications to the various functional groups present in them. ROS-induced oxidative modifications of biomolecules change their physicochemical properties, such as conformation, structure, solubility, reactivity, binding, proteolytic susceptibility, and enzyme activities. For example, 8-hydroxy-2-deoxyguanosine (8-Oxo-dG), an oxidized-derivative of deoxyguanosine (DNA), tends to show higher accumulation in aged tissues. Similarly, side chains of amino acid residues such as arginine, lysine, and proline undergo oxidative modification called protein carbonylation, a type of protein oxidation found to be highly accumulative, and reflective of cellular oxidative stress. Such chemical alteration and oxidation render these molecules nonfunctional and cause their accumulation, thereby decreasing the ratio of cell's overall functional-to-oxidized biomolecules, and consequently compromising the cellular functions.

The cellular ROS level may have different implications depending on the type of cells. For example, relatively increased ROS has been found to prolong the lifespan in *C. elegans* and yeast while it might have devastating effect on other types of eukaryotic cells. Under normal physiological conditions, ROS plays an important role in differentiation of hematopoietic stem/progenitor cells in *Drosophila* [19]. Generally, stem cells of various types and origins are differentially susceptible to damage due to elevated ROS level. Besides, experimental evidence has shown that stem cell ROS sensitivity also depends on the age of donor. For example, bone marrow and adipose tissue-derived mesenchymal stem cells (MSCs) isolated from aged donor show correspondingly increased susceptibility to ROS-induced oxidative

damage [20, 21]. Moreover, there has been direct correlation between ROS level and aging in HSCs, which may damage the replicative potential and leads to their exhaustion. Therefore, containment of ROS level by overexpression of superoxide dismutase (SOD) in either stem cells or their supporting cells has been proven to prolong the stem cell functions. There are several intricately connected networks, involving forkhead box O (*FoxO*) transcription factors that are responsible for regulation of cellular oxidative stress in various stem and non-stem cells. *FoxO* transcription factors play very important role in global metabolism, proliferation, and oxidative stress by modulating the expression of a battery of genes encoding antioxidant enzymes and proteins. Therefore, they play very significant roles in maintaining appropriate oxidative state as the deletion of *FoxO1*, *FoxO3*, and *FoxO1* leads to increased ROS level in HSCs and other stem cells. This elevated ROS level, if not reduced by treatment with antioxidant such as N-acetyl-L-cysteine, depletes HSCs and neural stem cells [22]. Besides, there are several mechanisms and signaling pathways involved in regulating oxidative stress, such as polycomb family chromatin regulator and DNA damage signaling molecule ATM, and thereby maintains pool of stem cells in various tissues [23].

Apart from ROS, stem cells are also highly sensitive to cellular level of molecular oxygen (O_2) and abnormal mitochondrial functioning. The oxygen sensitivity in stem cells is accomplished by hypoxia-inducible factor 1α (*Hif1α*), a transcription factor that plays a very crucial role in stem cell function, maintenance, and aging. Under normoxic condition, the cellular level of Hif1α is kept low by its continuous E3 ubiquitin ligase, von Hippel Lindau (VHL)-mediated ubiquitination and proteosomal degradation. However, hypoxia causes stabilization of Hif1α which, in turn, activates transcription of a range of hypoxia survival genes such as, glucose transporter, heat shock protein (HSP), and glycolytic enzymes. Several HSCs and neural stem cells (NSCs), which are naturally and anatomically located in hypoxic microenvironment, have had relatively stable Hif1α for their maintenance and survival. That is why the dentate gyrus NSCs and HSCs, deficient for Hif1α, rapidly deplete during aging. Surprisingly, overstabilization of Hif1α does not rescue HSCs either, rather impedes their function, indicating the need of precise control of Hif1α level for stem cell maintenance and functions [24].

Mitochondrial dysfunctions can occur due to multiple factors, including error-prone mitochondrial DNA polymerase, abnormal constituent protein in mitochondria, increased ROS level, and impaired biogenesis. Mitochondrial DNA (mtDNA) is relatively more susceptible to mutation and deletion-based alteration as it lacks association of protective histones and lesser capability of DNA damage repair compared to nuclear DNA. Comprehensive analysis of single cell-derived mitochondria shows domination of state of homoplasmy over heteroplasmy, that is, simultaneous existence of both mutant and wild-type genomes within the same cell, as a function of age. This shift in homoplasmy-to-heteroplasmy status is dominated by mutant mitochondria over normal mitochondrial genome, considerably increasing the mutational load in aging cells. Careful consideration of available findings shows aging in cells is predominantly induced by erroneous mtDNA replication. There is a functional correlation between mitochondrial dysfunctioning and aging-associated

phenotypes. However, mitochondria have also evolved certain protective and defensive mechanisms over a period of time to neutralize the oxidative damages to certain extent. For instance, to avert ROS-induced oxidative damage, mitochondria activate a range of ROS-detoxifying enzymes such as superoxide dismutase (SOD) and GPx1 with the help of peroxisome proliferator-activated receptor gamma co-activator 1 (PGC-1). In addition, PGC-1 promotes oxidative phosphorylation and mitochondrial biogenesis. This empirical evidence is further supported and strengthened by the fact that overexpression of PGC-1 delays the onset of age-related changes in intestine, increases tissue homeostasis and, more importantly, lengthens the lifespan in model organism like *Drosophila* [25].

Telomere Dysfunction

There is a direct correlation between extent of DNA damage and age, which, in turn, compromises genomic integrity and functionality. Though DNA damage can occur over any portion of chromosomes, however, some distinct regions on chromosomes are more susceptible compared to others. For instance, age-related chromosomal deterioration/attrition is more profound over the repetitive TTAGGG terminal ends of each chromosome, referred to as telomere. Telomeres protect genetic information and chromosome terminals from erosion and damage but they are shortened a bit each time cell divides as replicative DNA polymerases lack the capacity of replicating the chromosomal ends. Though nature has endowed each cell with a gene encoding the specialized reverse transcriptase, called telomerase for this function, but it expresses only in few cell types, including embryonic and adult stem cells. This leads to a steady and progressive loss of telomere-protective sequence located at either ends of each chromosome. The immediate question comes to mind, "why telomere shortening and/or breaking is not followed by the repair by DNA damage repair machinery? The reason being that each chromosome's telomeres are protectively occupied by a distinct multiprotein complex called as "shelterin." The main function of which is to deny DNA repair machinery access to telomeres. Otherwise, telomeres would be "repaired" immediately after DNA breaks leading to chromosome fusion and even more harmful consequences [26]. Heterozygous telomerase mutations potentially cause defect in organ regeneration and cancer development in humans. Telomere exhaustion underlies in vitro restricted proliferative potential that leads to replicative senescence or "Hayflick limit" [27]. Patients with telomerase deficiency or short telomeres are likely to develop aplastic anemia, dyskeratosis congentia, cirrhosis, and pulmonary fibrosis. Importantly, telomere shortening also precedes normal aging both in mice and in human.

Dynamic telomere length in cells is determined by ratio of functional telomerase to mitotic division-dependent telomere erosion. Unlike adult somatic cells, embryonic and adult stem cells have functional expression of telomerase that resist the telomere shortening as they keep elongating telomeres, albeit, to a certain and limited extent, making them better at balancing this ratio for longer duration. However, as a result of aging, telomeres shortening occurs even in the various stem cells,

restricting their proliferation-based regenerative potential in aging individuals. For example, age-dependent telomere attrition occurs in HSCs and intestinal epithelium cells, which is further accelerated in case of chronic diseases. This is possibly linked to decreased regenerative potential, tissue malfunction, and increased incidence of diseases in older human population worldwide. Telomere shortening in HSCs results into accelerated aging-based imbalance in HSCs pool due to alteration in stem cell environment and differentiation-inducing checkpoints, which lead to the loss of self-renewing lymphoid-biased HPSCs [28]. Typically, HSCs aging is characterized by prevalence of myeloid-biased HPSCs over lymphoid-forming HPSCs. This age-dependent lineage biasness in HPSCs results in increased myeloid cells at the expense of lymphoid cells, and is likely to cause increased susceptibility to infections and other diseases (Fig. 12.3). In addition, telomere dysfunction induces defects in mRNA splicing which leads to a cascade of molecular events responsible for strong positive selection during human aging [29].

Epigenetic Alteration

Epigenetics refers to heritable trait which results from chromosomal changes without alterations in DNA sequences per se. Epigenetics determines the regulation of genes, and is involved in many cellular processes. Epigenetic-based gene regulation precisely controls the fate of cells to a great extent and is one of the several mechanisms responsible for cellular differentiation, and hence, formation of various types of cells such as neurons, liver cells, heart cells, pancreatic cells, and so on. Such alterations in the epigenome have been found to be age-dependent. DNA methylation, that adds methyl group to DNA, is one among several ways to bring about epigenetic changes. Comprehensive studies aimed at such chemical addition have shown hypermethylation both globally and at CpG islands in normally aging tissues. Upon enforced proliferation, HSCs show global hypomethylation, indicating the possibilities of either hypermethylation preceding HSCs aging or vice versa [30]. Aged mouse HSCs show unusual DNA methylation of genes involved in self-renewal and differentiation, imparting aging-characteristic phenotypes. Moreover, some distinct CpG islands undergo increased methylation as individual ages, which leads to myelodysplastic syndrome (MDS), and eventually to acute myeloid leukemia (AML) [31]. The alterations in epigenome are accomplished by multiple types of epigenetic regulators such as DNA methyl transferase (DNMT), Tet methylcytosine dioxygenase 2 (TET2), and additional sex combs-like 1 (ASXL1) among others. Meticulous analysis of reported works invariably shows mutations in the abovementioned epigenetic regulators underlie the earliest genetic changes in neoplastic progression. A recent study in mice showed enhanced self-renewal and impaired differentiation of HSCs following biallelic knockout of DNMT3a, a family member of DNMT, which predisposes them to hematological disorders such as MDS and AML [32]. This suggests that the presence of functional DNMT3a suppresses the set of self-renewal genes, including β-catenin and Runx1, by methylation and thereby regulates several cellular processes [33].

Aging of skeletal muscle is characterized by reduction in mass and strength of muscle owing to quantitative and qualitative deterioration and decline in contractile myofibers [34], and, therefore, substantial reduction in its intrinsic regeneration potential [35]. Under normal physiological conditions, new muscle tissue formation occurs following fusion of cellular progenies formed by asymmetric division in muscle stem cells (MuSCs), also popularly known as "satellite cells" owing to their sublaminar location and juxtaposed association with the plasma membrane of myofibers. The remaining half of progenies constitutes the pool of muscle precursor cells. However, aging causes diminution of such muscle stem cell pool. The reason for depletion in muscle stem cells pool encompasses age-dependent cellular senescence, impaired self-renewal, or death. These mechanisms, leading to MuSCs decline, depend on both intrinsic as well as extrinsic factors as evidenced from partial restoration of proliferation and differentiation capacities following exposure to young environment or to growth factors. In addition, it also suggests the likelihood of reversal of aging-induced cessation of self-renewal and differentiation potential [36]. During human myoblast quiescence, methylation suppresses sprouty1 pathways, involved in quiescence regulation. MuSCs, isolated from old mice, showed elevated repressive H3K27me3 marking on histone proteins genes, which would otherwise remain unmethylated in younger counterpart. A recent study has shown existence of different epigenetic stress response in satellite cells isolated from young and aged mice. Aged mice were found to have drastic induction of active chromatin marks both at site-specific and global locations, resulting in specific induction of Hoxa9 gene. The Hoxa9 gene, in turn, leads to activation of satellite stem cell function through activation of various pathways, including TGFβ, JAK/STAT, Wnt, and senescence signaling, indicating altered epigenetic stress response in activated MuSCs, and the consequent limited satellite stem cell-based muscle regeneration characteristic of aged muscle [37].

Age-Dependent Enhancement in Replication Stress in Stem Cells

Replication stress is highly complex nuclear phenomenon with wide range of effects on genome stability, cellular proliferation, and differentiation, resulting in multiple human diseases. Replication stress response could be triggered following multiple changes such as generation of single-stranded DNA containing aberrant replication fork structure, aneuploidy, chromosomal instability (CIN), genomic instability (GIN), and so on. Replication stress is predominantly mediated by the kinase ATM and Rad3-related (ATR) pathways. Adult stem cells are sensitive to replication-based stress, which is further compounded in wake of rapid demand for cell division. Therefore, replication stress and DNA damage triggered by multiple ways, including burst of oxidative stress, leads to accumulation of genetic alterations and exponential increase in genomic aberrations in aging stem cells. There are various underlying causes with varying implications and effects, including nicks, gaps, stretch of ssDNA, unrepaired DNA lesions, short hairpins, and DNA triplexes, among others. A recent study found increase in phosphorylated form of the variant

histone H2AX (γH2AX) foci, indicative of DNA damage, as a function of time. Similarly, there is a direct correlation between age and DNA break as was shown using alkaline comet assay on the basis of experiments performed in purified HSCs from animals of different age groups [38]. Quiescent HSCs show high accumulation of all sorts of DNA damages compared to their proliferating counterparts owing to attenuation of DNA damage response and repair pathways. The underlying reason is aging-induced lower expression of mini-chromosome maintenance (MCM) helicase components. Furthermore, this leads to compromised HSC functions in response to various hematopoietic stresses, such as extensive blood loss and pathophysiological inflammation. Such stresses demand compensatory and dramatic increase in proliferation of quiescent or slow-proliferating HSCs, which is likely to result in increased DNA damage, and eventually, bone marrow failure [39]. One of the mechanisms leading to clonal dominance of mutant HSCs is thought to be replication stress, leading to higher susceptibility to developing diseases.

Aneuploidy has had several consequences on aging of stem cells. One such consequence is telomeric replication stress which, in turn, causes DNA damage at telomeres and consequent p53 activation. Telomerase-deficient mice show p53/RB-dependent depletion of hematopoietic stem cells. Contrary to this, endogenous telomerase expression in HSCs ensured alleviation of aneuploidy-induced replication stress and others. Therefore, telomerase plays a very crucial role in rescuing murine HSCs from aneuploidy-induced replication stress at telomeres and aneuploidy-induced senescence (AIS) and cell depletion, suggesting its suppressive role in telomere dysfunction-induced CIN, on the one hand, and ensuring replicative potential in aging stem and progenitor cells, on the other [40].

Age-Induced Shift in Proteostasis Equilibrium Drives Stem Cell Aging

Among multiple aging-inducing factors, impaired protein homeostasis, also known as proteostasis, has very important role to play as misfolded proteins can form toxic aggregates, disrupt membrane system, and thereby cause cell death and diseases. Under normal physiological conditions, protein synthesis occurs in a precisely controlled and concerted fashion through spatiotemporal control of ribosome biogenesis, recruitment, and loading, leveraging array of quality control molecular mechanisms. Proteostasis encompasses stabilization of correctly folded proteins, on the one hand, and removal of misfolded, damaged, aggregate, and unneeded ones through proteosome-based degradation, on the other [41]. However, with aging and age-induced extrinsic and intrinsic molecular alterations, there is an untoward shift in equilibrium leading to deranged proteostasis, which poses great risk of developing age-related diseases, such as Parkinson's disease, Alzheimer's disease, diabetes, cataracts, and Huntington's disease, among others [42, 43].

So, what does maintain proteostasis? There are two important global mechanisms employed by cells in order to maintain functional status of proteome in the cells, namely, chaperone-mediated protein folding and stability, and proteolytic

system. Chaperones are protein molecules that provide assistance to proteins and other biomolecules at the various levels, including folding, unfolding, assembly, and disassembly, as well as prevent newly synthesized polypeptide chain to form nonfunctional aggregate with preassembled subunits. Heat-shock protein family is one type of cellular chaperone that responds to stress-induced protein denaturation. However, this weakens and impairs substantially during aging in various cells, including aged stem cells [44]. As a result, accumulation of damaged (carbonylation and glycation) and misfolded proteins increases in aging individual, also suggestive of decline in capacity to maintain protein homeostasis. Overexpression and upregulation of chaperone (heat shock proteins) and co-chaperone have been found to considerably lengthen the lifespan, indicating their potential involvement in proteostasis, hence conferring functional dynamism at both cell and organism levels [45]. Moreover, transcriptional activation of transcription factor HSF-1, master regulator of heat-shock response, has been found to increase the thermo-tolerance and longevity in several nematodes [46]. Similarly, in mammalian cells, transactivation of heat shock genes, including Hsp70, was found to be considerably enhanced following deacetylation of HSF-1 by SIRT1, and downregulation of SIRT1 expectedly reduces the response [47]. In addition, pharmacological induction of Hsp72 has been found to delay progression of dystrophic pathology and preserve muscle function in mouse model of muscular dystrophy, suggesting another approach to restore proteostasis by activating protein folding and stability [48].

Cellular protein quality control is accomplished through proteolytic system, which encompasses two components, namely, ubiquitin proteasome system and the autophagy–lysosomal system. However, the working of this system declines with aging. Treatment of human cultured cells with either proteasome activator or deubiquitylase inhibitors enhances the clearance and disposal of toxic proteins [49], and, in yeasts extends the replicative life. Similarly, in nematodes, epidermal growth factor (EGF) signaling-induced expression of ubiquitin–proteasome system increases the lifespan [50]. Autophagy plays very important role in maintaining protein homeostasis as evidenced from improved hepatic function in transgenic mice expressing an extra copy of the chaperone-mediated autophagy receptor LAMP2a. Similarly, induction of autophagy by regular administration of mTOR inhibitor, rapamycin, has lifespan-extending effects on yeast, flies, nematode, and mice [51]. Deletion of Atg7 or Fip200, involved in autophagy, causes rapid depletion of HSCs, indicating the crucial role of autophagy in HSCs maintenance [52]. FoxO transcription factor transcriptionally activates the expression of chaperone and thereby helps promote the longevity and stem cell functions. Therefore, all the above evidence suggests that perturbed proteostasis can further precipitate age-associated risk and pathology and, therefore, leads to several degenerative diseases, which can be controlled by developing deeper insight-based intervention strategy.

Nutrient Sensing and Changes in Nutrition Affect Stem Cell Functions

Ever-expanding research on interrelationship between nutrient and stem cell functions has shown lots of promising results. Caloric restriction, that is, substantial reduction in food intake without causing malnutrition, has been found to be helpful in delaying onset of age-associated degenerative diseases and extends lifespan, partly by influencing the function of stem cells. For example, in a rodent model study, caloric restriction was found to enhance proliferation of progenitor cells, increase survival of newly formed astrocytes and neurons, and thereby promote neurogenesis in the dentate gyrus [53]. Extending caloric restriction study in *Drosophila* has shown similar result, wherein reduction of age-associated germline stem cells was found to be reduced. The underlying mechanisms of age-lengthening effects of nutrients through stem cell-dependent functions are yet to be clearly elucidated. However, reasons underlying the nutrient-induced changes may be the expression of systemic factors which, in turn, regulate stem cell functions as is evidenced from loss of intestinal stem cells and male germline stem cells following protein starvation in *Drosophila.* Reduction in stem cells occurs because of decreased expression of insulin-like peptides in brain, which can be overcome by constitutive expression of active insulin receptor, indicating direct involvement of insulin in maintenance of germline stem cells [54].

Among multiple pathways underlying the calorie-induced beneficial effects, target of rapamycin (TOR) signaling plays a very crucial role. The downstream effect of TOR signaling is protein synthesis and cell growth. TOR, a conserved serine/threonine kinase, is activated by multiple factors, including amino acids, nutrients, growth factors, etc. [55]. Reduced TOR signaling may slow down aging and extends lifespan of organism, probably by increasing the proliferation and functioning of stem cells of various organs. However, further study would be needed to make conclusive statement in this regard.

Ex Vivo Stem Cell Aging

Aging of stem cells in vitro reflects the process of in vivo aging to a large extent, especially with respect to phenotypic features and molecular mechanisms. The mechanism underlying in vitro aging of stem cells shows a lot of species-specific and individual-specific variations. For instance, telomere shortening drives cellular senescence in cultured human cells, which is not reported in rodent cells following the trajectory of replicative senescence [56]. Almost all types of cells, including stem cells, irrespective of their source of origin, undergo aging during culture, called ex vivo aging. Expansion of cell through in vitro culture is limited to a certain number of cell division due to replicative senescence. Thereafter, cultured cells undergo cell cycle arrest, increases in size, and acquire “fried egg” morphology. Comprehensive molecular analysis of such cells shows aberrant alteration with respect to transcriptomics, epigenomics, and secretory profile, suggesting

usefulness of replicative senescence for quality control of cell preparation for downstream therapeutic application [57]. Replicative senescence is, among others, induced by ever-changing DNA methylation landscape over the course of culture. However, it is not yet known as to what regulates age-specific methylation pattern, and how much significant role they play?

Mesenchymal stem cells show increased aging phenotype, such as decline in proliferation potential due to replicative senescence, downregulation of self-renewal-associated genes, such as Oct4, Sox2, and TERT, increased tendency for osteogenic differentiation, following repeated passage and long-term culture [58]. The actual age of culture is relied upon population doubling (PD). MSCs, derived from single-cell-based colony, can be expanded up to as many as 50 PD in about 10 weeks, which starts showing signs of senescence thereafter. The duration of PDs increases with increase in cell passage, suggesting decrease in proliferative potential as cell ages. The physical characteristics, reflective of cell aging such as cytoplasmic granules and debris, also increase with age of cultured cells. Telomere shortening usually occurs at the rate of around 50 bp over each passage which might differ according to type of cultured stem cells, medium, growth factors, etc. Telomere shortening destabilizes chromosome integrity, which affects expression dynamics of several genes. Cells at latter passages have also been found to have compromised lineage differentiation potential. In addition, the rate of senescence of stem cells during culture also depends on the age and health of donor. Aged in vitro cells also show substantial shift toward cancerous cellular morphology such as morphological transformation of elongated and adherent cells into round, nonadherent type. For instance, human adipose tissue-derived MSCs show spontaneous transformation into small, clustered aggregations, displaying chromosomal aberrations at the rate of around 50% [59]. In addition, various types of stem cells, including bone marrow–derived endothelial progenitor cells (EPCS), show decrease in clonogenicity and increased tendency to acquire round-shape morphology with aging [60]. There are various ways of measuring cellular senescence such as β-galactosidase assay, measuring level of senescence-associated genes, p21 and p16 among others. Although, these methods reveal and help quantify cellular senescence but they fail to unveil the underlying cellular mechanisms, leading to cell cycle arrest and cell senescence. Therefore, considering all the findings, ex vivo cell culture model could be tremendously useful in deepening our understanding of molecular pathways, and designing appropriate aging-lowering clinical intervention and reducing age-associated risk factors and pathologies.

Exercise Induces Stem Cell Functions and Slows Down Aging Process

Regular physical exercise has been proven to have many beneficial effects on functioning of tissues and organs by promoting the activities of resident stem cells. For example, neural stem cells were found to increase and so does the cognitive parameter, including learning and memory, following exercise in an experimental study

involving mice and human [61]. In addition, an experimental group of voluntarily running mice showed stimulation of cell proliferation and consequent neurogenesis in the hippocampal region of the brain. Moreover, exercise also induces the number of neural stem cells apart from neurogenesis in subventricular zone of forebrain. This physical exercise-induced stem cell stimulation, proliferation, and functions are mediated by multiple factors and corresponding signaling pathways. In a rodent study, expression of insulin-like growth factor-1 (IGF1) and growth hormone were substantially activated following exercise, which upon binding to their respective receptor may induce the beneficial neurogenesis activity as the same effect was not found in growth hormone-deficient mice [62]. IGF1, mainly produced by liver, is actively taken up by specific group of neurons, resulting into their proliferation and adaptive responses, which can be blocked by administration of antibody against IGF1. Even subcutaneous administration of IGF1 is found to be sufficient for neurogenesis in dentate gyrus [63]. Considering aforementioned empirical evidences, physical exercise may play very important role in activating the stem cell functions and thereby slow down aging process.

Role of p53 in Aging of Stem Cells

Among tumor suppressor genes, p53 has been known to play very important role in a range of cellular activities, including cell cycle arrest and apoptosis. Throughout life of organisms, there is an accumulation of various forms of DNA damage owing to generation of reactive oxygen species (ROS), exposure to a range of potential mutagens, error-prone DNA replication, etc. These damages, if left unrepaired, may lead to several diseases such as cancer. However, our cells are endowed with a range of reparative potential which is mediated through p53-dependent mechanisms. Depending upon extent of DNA damage, p53 triggers different responses which would either repair the damage or lead the cell on the path of senescence and cell death, and that is how it helps preserve the genomic integrity and resist the development of diseases. In addition, p53 is involved in a range of very crucial cellular activities, such as cell cycle regulation, maintaining conducive cellular redox state, and various metabolic processes. The significance of DNA repair process can be observed in case of segmental progeria syndrome—rare human disease characterized by premature aging phenotypes such as skin atrophy, cataracts, osteoporosis, heart diseases, cerebellum degeneration, hair graying, immunodeficiency, cancers, and consequent reduced lifespan. This is triggered off by impaired DNA repair processes owing to loss of function in DNA damage signaling protein ATM and RecQ DNA helicase WRN [64]. Furthermore, loss of DNA damage signaling protein, ATM has been implicated in depletion of HSCs and heightened loss of melanocyte stem cells following low dose radiation. There has been similar and consistent progeroid phenotypic display and accelerated aging in mice carrying mutated genes associated with human progeroid syndromes [65], further reinforcing and

consolidating the empirical evidences, which suggest unrepaired DNA damage as a fundamental cause of aging. Moreover, mutation in p53 is a very commonly observed phenomenon in various types of cancer, indicating its pervasive role in growth, development, and diseases [66].

Apart from being involved in normal cells, p53 also plays a very crucial role in promoting stem cell-based tissue regeneration, repair, and homeostasis by maintaining functional genomic integrity. For example, p53 has been found to regulate cell division, differentiation, and chromosomal stability in mouse olfactory bulb stem cells [67]. In addition, p53 has been found to regulate cell division polarity by restoring asymmetric division and, thus, the self-renewing potential of mammary stem cells. Similarly, there are other types of stem cell and progenitor cell populations whose proliferation and differentiation are regulated in a similar manner [68–70]. Therefore, p53-mediated DNA repair and other regulatory pathways help restore DNA damage, maintain stem cell repertoire, and thereby delay cellular aging along with substantial reduction in the rate of organismal aging (Fig. 12.4).

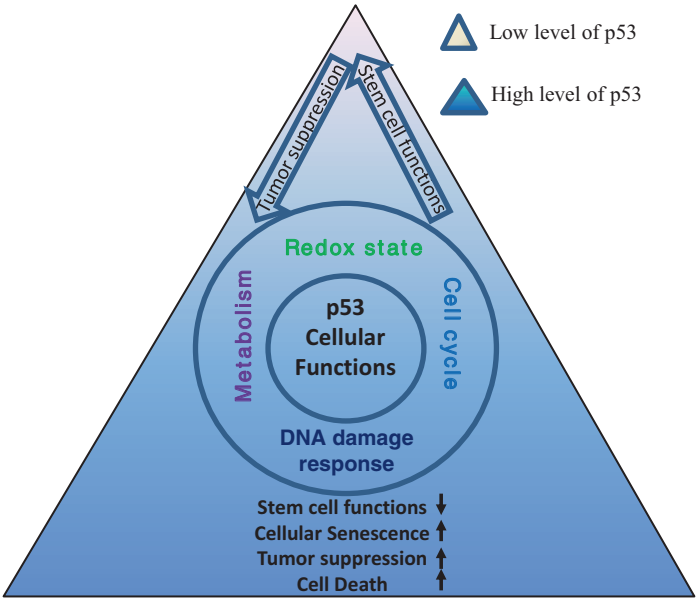

Fig. 12.4 Cellular expression of p53 and its effect on various stem cell functions. P53 plays a very crucial role in many vital cellular functions such as cell cycle regulation, metabolism, maintaining conducive redox state, and DNA damage response, among others. Elevated level of p53 (bottom of pyramid) represses stem cell proliferative functions while inducing cellular senescence, tumor suppression, and cell death. On the other hand, its lower expression level promotes stem cell proliferation and associated functions at the cost of increased incidence of tumorogenesis (top of pyramid)

Discussion and Conclusions

Aging is characterized by a gradual decline and deterioration in tissue homeostasis, which is attributed to age-associated impairments in tissue function, intrinsic regeneration, and developmental potential. Generally, tissue homeostasis is maintained by balancing the ratio of tissue damage to tissue repair, leading to continuous renewal of structural and functional aspects of organs over time. Under normal physiological conditions, adult stem cells, residing in various tissues and organs, carry out such renewal and repair processes as and when need arises. Although, there is no doubt whether age-dependent decline in tissue regenerative potential occurs, but an important question arises—is it because of intrinsic aging of stem cells or impairment of stem cell function in aged microenvironment or both? Answer seems both ways considering all the empirical evidences. Unraveling the underlying molecular mechanisms and their functional integration will be critical in designing stem cell-based therapeutic applications with regard to aging intervention, tissue injury, and age-associated degenerative diseases. Though there are a lot of commonalities regarding molecular pathways and their regulators between stem and non-stem cells, many of them are unique to former only. This suggests that underlying aging mechanisms in various cells is similar on many accounts and some are unique to stem cells only. For instance, common hallmarks of aging phenotypes both in stem cells and in non-stem cells include ROS production, DNA damage, telomere attrition, proteotoxicity, and aberrant changes in epigenetic landscape, among others.

Over the past decades or so, there have been tremendous progresses in understanding of mechanisms underlying the molecular control of stem cell functions during normal and pathophysiological conditions. Furthermore, emerging empirical evidences from range of stem cells distinctly unravel prevalence of many overlapping mechanisms and their differential regulations in different microenvironments during the aging. This ever-expanding multidimensional knowledge on interrelationship of stem cells and aging will help us in strengthening the currently existing regime of clinical intervention in aging as well as designing new treatment which would help improve the stem cell-based tissue homeostasis and regeneration and thereby reduce the incidence of age-associated diseases in the ever-expanding aged human population worldwide.

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