Current understanding of the mechanisms of stem cell aging

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Abstract Remarkable progress has taken place in the research of stem cells during the past 30 years. General perceptions, experimental and clinical evidences pinpoint the fact that functional decline of tissue often coincides with aging-related diseases. Stem cell aging plays a fundamental role in the dysregulation of tissue function, maintenance, and repairing. This review specifically focuses on the current findings and emerging concepts of hematopoietic stem cell aging and its mechanisms.

Keywords Aging; Hematopoietic stem cells; Mitochondria; Metabolism; Epigenetic regulation
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1. Introduction

Individual stem cells face three major choices: to divide (mitosis), to specialize (differentiation), or to commit suicide (cell death). The balance between these processes ensures that the cell number in an organism remains essentially in functional equilibrium (homeostasis). While mitosis, differentiation, and cell death have received quite a lot of attention for dozens of years, the fourth choice, to age, has become a research hotspot in the last decade [1]. Until recent times, reports on stem cell aging have emerged focusing on physiological aging, tumor suppression, cell cycle arrest, DNA replicative stress/damage response, etc. Now, we know that stem cell aging, at least in part, is due to intrinsic (e. g. telomere shortening) and extrinsic factors (e. g. energy deprivation) which deteriorate stem cell niche and/or stem cell *per se* [2—7] (Figure 1). We also know many pathways regulating stem cell aging, taking hematopoietic stem cell (HSC) aging for example, is accompanied by increasing levels of noncanonical Wnt5a signaling, which can be modulated to functionally rejuvenate HSCs via repression of Wnt5a expression [8].

In retrospect, it is surprising that for a long time biologists seldom questioned the fate of stem cells in our body. Nevertheless, recent studies implicate the possibility of rejuvenating aged cells via activation of certain transcription factors [9]. For example, Sox2, a key mediator to maintain cell pluripotency and inhibit differentiation, was found the most upregulated transcription factor in the cancer stem cells [10], but senescence deregulates its expression [11]. This led to intensive efforts to silence tumor seeds by inhibiting such pluripotency factors [12], while also intrigued a thought another way round—how to rejuvenate the stem cells in old tissues without triggering malignant diseases? The vast commercial benefits and the lack of effective anti-cancer drugs account for a proportion of the reason, an impenetrable barrier to elucidate the mechanism of stem cell aging and regulation of tissue homeostasis (stem cell aging *vs.* tumorigenesis) impedes the seeking of desirable elixir of life.

Fortunately, because of the rapid growth of stem cell research, many targets and signaling pathways involved in the underlying mechanisms and regulation of stem cell aging have been identified, although some of the current studies are still controversial. Since much of what is known about stem cells has been

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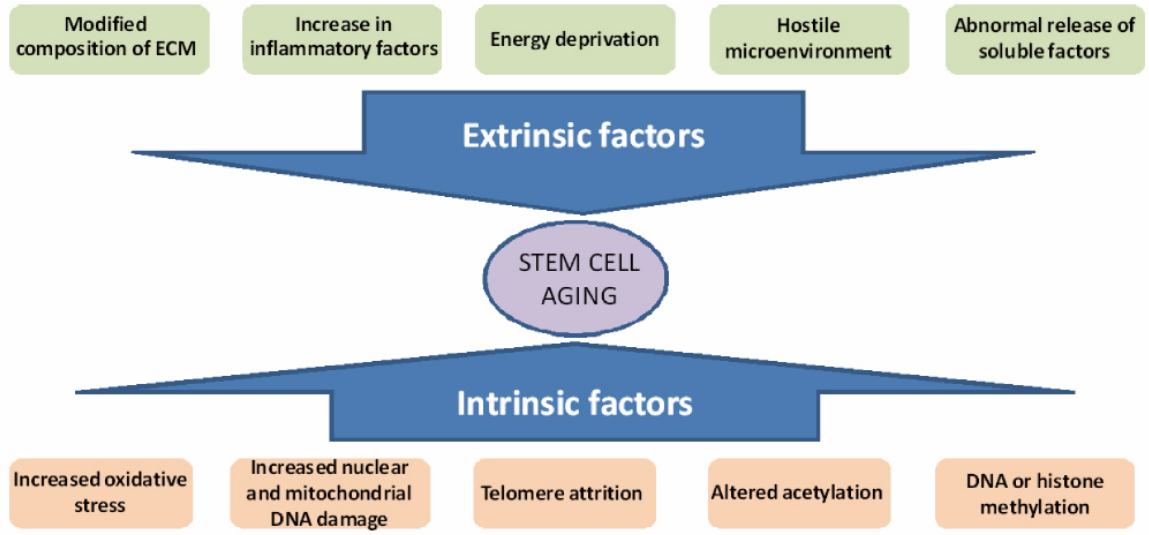

Figure 1 Intrinsic and extrinsic factors contribute to stem cell aging. ECM, extracellular matrix; DNA, deoxyribonuleic acid.

learnedfrom studies conducted on the HSC system, here in this review, we try to highlight those known and yet unexploited key molecular and signaling pathways susceptible to HSC and other stem cell aging as well. Potential therapeutic targets linking to tissue regeneration/homeostasis are also discussed.

2. Redox and metabolic regulation of stem cells aging

Different from somatic cells, stem cells were initially thought to be endowed with unlimited self-renewalcapacity, thus exempt from aging. However, increasing experimental evidence has demonstrated reduced self-renewal capability in aged HSCs using serial transplantation assays [13, 14]. When HSCs are transplanted into lethally irradiated young recipients, HSCs from aged donors exhibit significant reduction on efficiency in hematopoiesis and homing to bone marrow in comparison to HSCs from young donors [14—16]. One notion to such irradiation-induced HSC senescence indicates accumulation of toxic metabolites, e. g. reactive oxygen species [ROS], whose accumulation is generally considered deleterious to stem cell function in the context of aging [14, 17]. Indeed, exposure to ambient oxygen leads to impaired recovery of long-term repopulating HSCs, while pharmacological inhibition of such oxygen stress could increase the amount of transplantable HSCs [18]. On the other hand, stem cell senescence has been proven to be crucial to stem cell dysfunction, therefore a potential target to combat against. Very recently, a 'senolytic' pharmacological agent (ABT-263, an inhibitor of the anti-apoptotic proteins BCL-2 and BCL-xL) has been found to selectively kill senescent cells, thus mitigating irradiation-induced premature aging of the hematopoietic system and rejuvenating the aged HSCs and muscle stem cells in normally aged mice [19]. Such senolytic drugs may represent a new class of anti-aging agents helping maintain redox and metabolic homeostasis.

It has been generally accepted that aging-induced mitochondrial dysfunction leads to boost of ROS production and reduced oxidative phosphorylation, therefore decreasing ATP synthesis and cell respiration. Mitochondria contain their own genome. It has been reported in many cell systems that mutations in mitochondrial DNA (mtDNA)accumulate during aging [20], with ROS as a principal cause [17]. However, it is very difficult to extract sufficient amount of mitochondria in stem cells for biochemical analyses due to the rarity of stem cells. Nonetheless, several studies have found the association of mtDNA defects with stem cell aging [21—23]. Interestingly, using a model carrying an error-prone form of the mtDNA polymerase gamma(POLG), Norddahl *et al*. have shown that intact mitochondrial function is required for appropriate multilineage hematopoietic cell differentiation, but not mtDNA mutations *per se* being a primary

driver of stem cell aging [24]. Peroxisome proliferator-activated receptor gamma coactivator 1alpha (PGC1α) is a pivotal regulator of mitochondrial biogenesis and function [25—27]. Profound repression of PGC1α has been seen in mice of telomere dysfunction, which leads to significant metabolic and mitochondrial compromise via p53 signaling pathways [28]. Another investigation reported that PGC1α protects from stress-induced depression using skeletal muscle PGC1α induction (via exercise or muscle specific overexpression), without the need to cross the blood-brain barrier [29]. Mitochondrial function of telomerase deficient (Terc-/-) iPSCs and their differentiated derivatives is severely impaired, while the mitochondrial function in Terc-/- ntESCs is considerably improved, with PGC1α a possible target [9]. Tissue-specific overexpression of PGC1α in drosophila stem and progenitor cells within the digestive tract extends life span [30]. Using the tissue-specific PGC1α overexpression mice model, our unpublished data unveiled multiple beneficial aspects of PGC1α coping with mitochondrial degeneration in the context of telomere deficiency. Though challenging, it is of great interest to replace compromised mitochondria with young ones via mitochondrial transplantation [31], thus slowing the aging process.

Yet the causal role of oxidative damage in the aging process remains controversial, in part because of the absence of a clear correlation between the efficacy of antioxidant defenses and extended cell function or longevity. Lack of human studies on toxic oxidative metabolites in tissue aging also makes scientists tiptoe cautiously at the crossroad. More recently, *Science* released a series of reviews discussing the role of ROS in cell signaling and homeostasis [32], suggesting mitochondrial ROS are not always deleterious and can even stimulate pro-longevity pathways. In addition, other study reveals that scavenging the ROS retards the differentiation of Drosophila haematopoietic progenitors into mature blood cells, indicating its role in priming haematopoietic cells for differentiation [33]. Nonetheless, studies on the genetically manipulated mouse model suggested that various metabolism-related pathways have been found to involve oxidative stress management and thus regulateHSC function and maintenance, including the Akt/FoxO transcription factors [34—36], the Pten/Akt/mammalian target of rapamycin (mTOR) pathway [37—39], Lkb1-AMP-activated protein kinase (AMPK) pathway [40], the ATM-BID pathway [41], Nrf2/Cxcr4 pathway [42], the sirtuin family [43—45], etc. Together, these studies suggest that aging of stem cells may be closely correlated with the metabolic and redox state of HSCs, which in turn can influence intracellular homeostasis of ROS and their functionalities.

3. Epigenetic regulation of stem cells aging

Epigenetic dysregulation represents an important hallmark of overall aging and contributes to aberrant stem cell function and tissue regenerative capacity [46]. Several epigenetic regulatory components, e. g. DNA methyltransferases (DNMTs), histone deacetylases (HDACs), H3K9 histone methyltransferases (Suvs) and others, have been implicated to function in the balance between self-renewal and differentiation in multiple adult stem cell compartments [47, 48]. For instance, epigenomic profiling of young and aged HSCs reveals concerted changes during aging that reinforce self-renewal and diminish differentiation [49]. Among those components, compelling evidence links the sirtuins as an anti-ageing family with stem cell aging [50, 51]. The muscle-selective absence of histone deacetylase SIRT1 mirrors the phenotypes of muscle stem cell aging, including reduced myofiber size and decayed muscle regeneration [52]. Similarly, inhibition of SIRT1 or SIRT2 recapitulates defects in nicotinamide phosphoribosyl transferase (Nampt) deficiency-induced neural stem/progenitor cell aging [53]. SIRT3is highly enriched in HSCs that regulates the global acetylation landscape of mitochondrial proteins and reduces oxidative stress. It was found essential under stress or old, but dispensable when young [45]. Intriguingly, the expression of SIRT4 increases while the expression of SIRT1 decreases in both differentiating and aging spermatogonial stem cells [54]. Moreover, SIRT7 expression was reduced in aged HSCs, and SIRT7 inactivation caused reduced quiescence, increased mitochondrial protein folding stress, and compromised regenerative capacity of HSCs [55].

Aside from (de-)acetylation regulation by sirtuin family, methylation status of DNA also changes with age[56], while deletion or forced expression of demethylases may affect lifespan [57]. Physiological aging

and experimentally enforced proliferation of HSCs both led to DNA hypermethylation [58], where aged HSCs exhibited broader H3K4me3 peaks across HSC identity and self-renewal genes and showed increased DNA methylation at transcription factor binding sites [49]. Loss of *de novo* DNA methyltransferase Dnmt3a results in HSC expansion and impaired differentiation, whereas loss of Dnmt3b resulted in that Dnmt3a-null HSCs can drive some differentiation and generate paradoxical hypermethylation of CpG islands [59]. Exome sequencing unveiled somatic DNA demethylaseten-eleven translocation-2 (TET2) mutations were specific to individuals with clonal hematopoiesis and were associated with alterations in DNA methylation [60], while in adult neural progenitor cells deficient in TET1, a cohort of genes involved in progenitor proliferation were hypermethylated and downregulated [61]. ASH-2trithorax complex, which trimethylates histone H3 at lysine 4, functions as a regulator of longevity in *C. elegans* [62]. Specific ablation of the atypical NOTCH ligand delta-like homologue 1 (Dlk1) imprinting in both NSCs and niche astrocytes is associated with postnatal acquisition of DNA methylation in the germ-line-derived imprinting control region [63]. Ezh2-mediated H3K27 methylation modulates HSC-specific genes such as Evi-1 and Ntrk3, aberrantly expressed in haematologic malignancies [64]. Recently, a global loss of H3K9me3 has been found during the differentiation of WRN-deficient embryonic stem cells to mesenchymal stem cells (MSCs), knock-in of catalytically inactive SUV39H1 in MSCs also exhibited premature cellular aging, mirroring those seen in WRN-deficient MSCs. Moreover, decrease in WRN and heterochromatin marks have been detected in MSCs from older individuals [65]. Thus, it is plausible that resetting the aged epigenome in stem cells may reverse aging phenotype and restore stem cell function.

4. DNA damage response in aged stem cells

Accumulated DNA damage in aged stem cells and other aging cell types is often accompanied by changes in the DNA damage response (DDR), where aged HSCs show increased replication stress [66] and compromised capacity to repair DNA damage [67—69]. Indeed, aged HSCs show an increased γH2AX-positive cell number [70], one possible reason is deficiencies in DNA damage repair limit the function of aged HSCs [71]. However, the level of DNA damage in stem cells should be evaluated with caution since such damage can be repaired [72] or involved in normal differentiation [73]. Nonetheless, aging-related alteration in DDR pathways of tissue-specific stem cells is a field of growing attention and investigation. The relevance and mechanism by which accumulation of DNA damage in aged stem cells may contribute to stem cell dysfunction are still under investigation.

One study has demonstrated that HSC quiescence and concomitant attenuation of DNA repair and response pathways underlies DNA damage accumulation in aged HSCs [72]. In contrast, repeated activation of HSCs out of their quiescent state induced attrition of normal HSCs [74]. Another notion suggests that-DDR ensuing loss of homeostasis in aging tissues might create a microenvironment that favors the selection of stem cells with higher self-renewal but also neoplastic transformation [75]. To counter the potential for malignant transformation, an important downstream consequence of activation of the DNA damage checkpoint is induction of cell cycle arrest signaling in response to cellular stresses, including telomere shortening [76—78]. Indeed, we previously reported the essential downstream effector of p53, p21; its deletion improved stem cell function and lifespan of mice with dysfunctional telomeres without accelerating cancer formation [79]. Interestingly, our recent study reveals a mechanism of stem cell aging, in which distinct effects of p53 and mTORC1 pathways on HSC aging are governed by Wild-type p53-induced phosphatase 1 (Wip1), which negatively regulates several tumour suppressor and DNA damage response pathways [80], but also B lymphocyte maturation and tissue regeneration [81, 82].

5. Energy metabolism in aged stem cells

Apart from DNA damage, energy crisis also threatens stem cell function normally displayed as aging-related metabolic defects or mitochondrial dysfunction in stem cells, which unbalance energy homeostasis

mainly via the nutrient sensing pathways [83—86]. In HSCs, an age-dependent decrease in nutrient uptake capacity has been observed, while Foxo3A-mediated autophagy is needed to mitigate energy crisis upon starvation [87]. The liver kinase B1 (LKB1)promotes HSC quiescence and metabolic homeostasis. Surprisingly, these effects on HSCs occur independently of AMPK/mTOR and FoxO signaling [40, 88, 89]. In addition, activation of the phosphoinositide 3-kinase-AKT pathway leads to inhibition of FoxO transcription factors, which balances oxidative phosphorylation and glycolysis in crosstalk with AMPK [90]. Intriguingly, a recent study reported that reduced levels of NAD+ contribute to the mitochondrial decay associated with skeletal muscle aging [91]. This reprogramming of cellular energy metabolism decreases intracellular NAD+ levels and the activity of SIRT1, leading to activation of muscle gene transcription [52]. Moreover, increased SIRT1 expression has been found to promote survival in a mouse model of genomic instability and suppress age-dependent transcriptional changes [92]. These data indicate that raising the sirtuins level could restore stem cell function in old tissues. It will be interesting to investigate the roles of sirtuins in regulation of NAD+/NADH ratio in age-dependent stem cell dysfunction of different tissues and species.

Recently, a controversial study using parabiosis demonstrated that the enhanced level or administration of GDF11 could correct DNA damage accumulated in aging mouse satellite cells [93], while its paralogmyostatin regulates energy homeostasis in the heart and prevents heart failure [94]. Although heterochronicparabiosis of young and old mice implies the potential to attenuate the aging process and improve stem cell functions, how to rejuvenate aged stem cells via autologous rather than allogeneic resources remains a central question to avoid issues regarding immunity and ethics. Calorie restriction (CR) represents a simple but also non-invasive approach to extend health span and ameliorate age-related pathologies at least, via enhancing the function of tissue-specific stem cells in a broad spectrum of animals. Many signaling pathways, such as SIRT1, AMPK, FoxO, and mTORC1, have been implied to affect stem cell function through their activation or inhibition [95—97]. However, no evidence to date indicates whether it also works in humans. The level of CR is indeterminate from individual-by-individual and long-term CR sounds impracticable to general public. In contrast, resveratrol, a naturally occurring small molecule that induces metabolic benefits resembling those of CR, increase mitochondrial biogenesis, in part through activation of SIRT1 and PGC1α [98]. Similarly, metformin, a hypolycemic agent widely applied in type II diabetes mellitus, has been found to extend longevity and health span in animal models [99, 100]. Rapamycin also exerts a similar protective response in *C. elegans* and mice [101]. Such endeavor is worthy trying that directs strategies to mimic the life-extending effect of CR using known compounds and pathways. Physical exercise also gives considerable benefits in improving energy metabolism, yet this is unsuitable to population with conditions, such as cardiovascular diseases, asthma, osteoarthrosis, diabetes, etc., which predominantly happen among the aged. Further elucidation of regulatory mechanisms of energy homeostasis in aged stem cells will be informative to anti-aging agent design and therefore intervening stem cell dysfunction during aging.

6. Conclusion

Stem cell functions decline with age because of unbalanced homeostasis which is triggered by an alteration in cell intrinsic properties (Figure 2). It is hard to place particular weight on any one pathway with respect to aging mechanisms, since the organismal aging is a complex process involving enormous cellular components from molecular to organelle alteration. In-depth and extensive exploration on key molecules and critical mechanisms of stem cell aging is therefore still very much sought after. Encouragingly, certain signaling pathways do appear more broadly involved than others, for example, we demonstrate that Wip1 regulates stem cell aging [80] and tissue regeneration [82] possibly via synergetic p53 and mTORC1 pathways, indicating a common mechanism for DNA damage response, stem cell aging, and tissue homeostasis.

Current knowledge directs us to maintain 'good seeds and soil' by balancing energy metabolism, redox

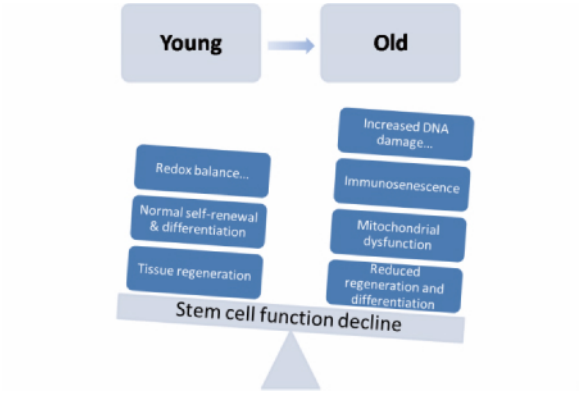

Figure 2 Factors trigger imbalance in normal stem cell function maintenance when getting old.

homeostasis, and other signaling pathways regulating transcriptome, epigenome, and proteome. Indeed, we have demonstrated DNA damage response is closely linked to dysregulation of stem cell metabolism, in which aging-related factors play a crucial role in the interactions. Emerging concepts such as to remove senescent cellsin aged tissue hold promise for rejuvenating tissue stem cells and extending health span. Screening of key molecules synergistically regulating a sort of common pathways (e. g. tissue regeneration, proliferation, DDR, immunity, etc.) is of great interest for strategic anti-aging and treating aging-related diseases. Such novel interventions open avenues for therapeutically applicable anti-aging drug development; meanwhile, we still need to continue fundamental investigations to understand the mechanisms of tissue specific stem cell aging, for a better and more specific regulation of tissue homeostasis when we become old.

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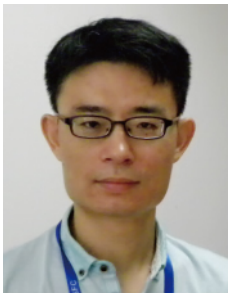

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