# Oxidative Stress-Mediated Stem Cell Aging

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**Abstract**

Stem cells, which are a class of cells with self-renewal capacity and multipotency of differentiation, play a critical role in tissue maintenance and regeneration in the whole life span of multicellular organism. With the recent researches about stem cell, it is realized that the aging and depletion of stem cells are closely related to organ aging and the occurrence of aging-related diseases, such as cardiovascular and cerebrovascular diseases, autoimmune diseases, and Alzheimer's disease. Thus, understanding the molecular mechanisms of stem cell aging will be important for developing new therapies for aging-related diseases. There are many intrinsic and extrinsic factors promoting stem cell aging. Oxidative stress has been recognized as the major cause of stem cell aging. Oxidative stress is the result of an excessive production of reactive oxygen species (ROS) and an impairment of the antioxidant defense systems. Excessive production of ROS and insufficient cellular antioxidant trigger a variety of aging-related pathways to induce stem cell senescence and aging. In this chapter, we summarized the biologic features of stem cell aging and discussed how oxidative stress affects stem cell aging and the main signal pathways of stem cell aging triggered by oxidative stress. We further explored how stem cells manage ROS accumulation and adapt to oxidative stress. Finally, we discussed the potential strategies against stem cell aging by controlling oxidative stress.

**Keywords**

Stem cells · Aging · Oxidative stress · ROS

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Stem cells are a class of cells with unique ability of self-renewal and multipotent differentiation capacity. In mammals, stem cells can be divided into two basic types: embryonic stem cells (ESCs) and adult stem cells (ASCs) [1]. ESCs originate from the inner cell mass of preimplantation embryos and exhibit both true pluripotency and self-renewal capacity. ASCs reside in variety of adult tissues (e.g., bone marrow, adipose, peripheral blood, liver, pancreas, hair follicle, skeletal muscle) and differentiate into almost all cell types of their originated tissue [2, 3]. Thus, ASCs are critical for maintaining tissue homeostasis and preserving regenerative capacity after injury. Emerging evidence demonstrated that age-associated imbalance of tissue homeostasis is at least partially caused by a loss of the regenerative capacity of ASCs linked to the accumulation of age-associated damage. During aging, there are various stress factors that regulate stem cell activity and promote stem cell aging. Oxidative stress caused by excessive production of reactive oxygen species (ROS) is one of the most significant endogenous toxins and inducer in stem cell senescence and injuries [4]. High-level ROS can trigger stem cell aging through directly inducing DNA, protein, and mitochondrial damage or accelerating telomere shortening. The mechanism underlying oxidative stress-mediated stem cell damage and senescence is a very complex signaling network. Meanwhile, stem cells possess several endogenous antioxidant stress strategies to manage ROS accumulation and adapt to oxidative stress. Therefore, understanding the complex signaling pathways mediated by oxidative stress as well as the antioxidant stress strategies of stem cell is very important to find targets for inhibiting stem cell aging.

## 3.1 Characteristics of Stem Cell Aging

All multicellular organisms exhibit declines in the function of tissues and organs with age, which is at least partially due to the loss of stem cell number and function over time. Stem cells in many tissues undergo obvious changes with age, exhibiting impaired self-renewal, abnormal proliferative activities, limited differentiation, and declined functional capacities [5]. These changes resulted in stem cell blunted responsiveness to cell replacement and tissue regeneration in old organisms. The mechanisms that underlie cellular aging are composed of intrinsic alterations and extrinsic alterations. Intrinsic alterations include telomere reduction, DNA damage, mitochondrial dysfunction, and loss of proteostasis. Additionally, extrinsic alterations can range from stem cell niche to systemic environment, such as oxidative stress, inflammation, and irradiation.

The characteristics and molecular changes of aged stem cell have been reviewed extensively elsewhere, which demonstrate the biology features of aged stem cell from different tissue are not uniform [5, 6]. For example, the number of functional hematopoietic stem cells (HSCs) decreases with age, and the differentiation potential of aged HSCs is also skewed toward the myeloid lineage at the expense of the lymphoid lineage [7, 8]. Similarly, the number and function of neural stem cells (NSCs) and melanocyte stem cells decline with age. However, the numbers of hair

follicle stem cells (HFSCs) and fly intestinal stem cells (ISC) do not change with age, but concomitant with a decline in function [9, 10].

## 3.2 Oxidative Stress-Mediated Signaling Pathways in Stem Cell Aging

Oxidative stress can be simply defined as excessive production of ROS in the presence of reduced antioxidants. ROS are composed by a diverse range of oxygen-containing entities which possess higher reactivity than molecular oxygen ($O_2$), such as superoxide radical anion ($O_2\cdot^-$), hydroxyl radical (·OH), and hydrogen peroxide ($H_2O_2$). Mitochondria are primary sources of ROS in most cells, a consequence of its role in energy production via oxidative phosphorylation (OXPHOS). During OXPHOS process, electron can leak prematurely to oxygen from the complexes I and III of mitochondrial electron transport chain [11]. Although mitochondria are primary sources of ROS in most cells, NADPH oxidases (NOX) are another major source of ROS. NOX consumes NADPH to generate $O_2\cdot^-$ and ROS.

Excessive ROS has been viewed as one of the leading causes of stem cell aging, which is attributed to the ROS-mediated oxidation of biologic macromolecules such as DNA, proteins, and lipids, which consequently promote cellular damage and senescence. The mechanism underlying oxidative stress-induced cell aging is involved in complex signaling networks regulated by several of regulators (Fig. 3.1), which are susceptible to redox changes and are recognized as redox

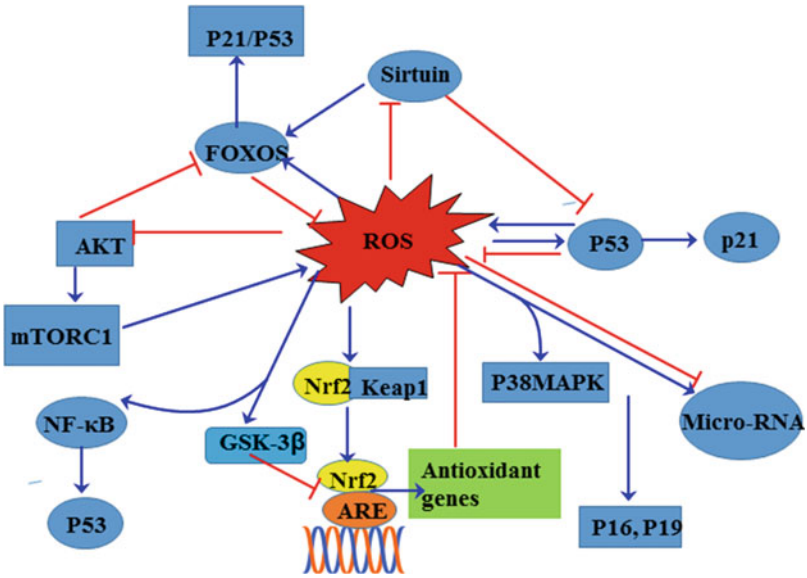


**Fig. 3.1** The signaling molecules involved in oxidative stress-induced stem cell aging

sensors. Thus, understanding the role of redox sensors in oxidative stress-induced stem cell aging is essential for development of the molecular basis for improving stem cell therapy efficacy.

### 3.2.1 p53 Pathway

p53 is a transcription factor that controls the expression of a range of target genes and plays critical roles in regulating cell cycle arrest, apoptosis, or senescence in response to numerous stress that lead to genomic instability, including oxidative stress. The previous studies present evidence that ROS act as an upstream regulator of p53 and p53 can in turn influence cellular ROS production. At low levels of oxidative stress, p53 induces antioxidant gene expression to attenuate oxidative stress and improve cell survival ability; by contrast, under high levels of oxidative stress, p53 shows pro-oxidative activities via upregulating the expression of pro-oxidative genes (e.g., p53-inducible gene 3 and proline oxidase), which further increases oxidative stress levels to induce cell death [12]. Furthermore, p53 exhibits pro-oxidative activities via suppressing the expression of anti-oxidative genes, leading to susceptibility to oxidative stress.

The previous studies reported that a constitutively active form of p53 induced a premature aging like phenotype in mice [13], while impairment of the activity of p53 ortholog CEP-1 extended life span of in *Caenorhabditis elegans* [14]. These results suggest that p53 activity is relative with aging. The aging process of mesenchymal stromal cells (MSCs) is often associated with activation of p53. More recent study reported that excessive ROS upregulated p53 expression, which binds to parkin and inhibits the biologic function of parkin to decrease the level of mitophagy, resulting in senescence of bone marrow MSCs [15]. In human umbilical cord-derived MSCs, it was also found that inhibition of p300 accelerates premature senescence via activation of p53/p21 signal pathway [16]. Wild-type p53-induced phosphatase 1 (Wip1) regulates HSCs aging via directly regulating p53 pathway, since Wip1-dedicicent mice exhibited multifaceted HSC aging phenotypes, while deletion of p53 can rescue the multilineage repopulation defect of Wip1($^{-/-}$) HSCs [17]. However, in skeletal muscle stem cells (MuSCs), it was discovered that the p53 activity was decreased with age, and pharmacological activation of p53 promotes the expansion of aged MuSCs in vivo [18]. Furthermore, genetically manipulated mice with p53 overexpression decreased aging-associated damage, which indicated that activation of p53 showed protective efforts to aging [19]. In summary, the role of p53 in stem cell aging is dependent on the levels of cellular oxidative stresses and cell type.

### 3.2.2 Nuclear Factor-E2-Related Factor 2 Pathway

Nuclear factor-erythroid 2-related factor 2 (Nrf2) is a ubiquitously expressed stress-responsive transcription factor, which regulates the expression of cyto-protective genes and antioxidant enzymes in stem cells. The activity of Nrf2 is tightly regulated

by Kelch-like ECH-associated protein 1 (Keap1). Under normal condition, Nrf2 is binding to Keap1 in the cytoplasm and subsequently degraded through the ubiquitin-proteasome pathway, which resulted in the low-level activity and expression of Nrf2 [20]. Under oxidative stress, high levels of intracellular ROS promote Nrf2 dissociating from Keap1 either by the oxidization of the reactive cysteine residues (Cys273, Cys288, and Cys151) of Keap1 or by activation of kinase, such as MAPK, PI3Ks, and PKC that phosphorylate Nrf2 [21, 22]. Then, the dissociated Nrf2 is transferred to the nucleus, where it dimerizes with the small Maf proteins and binds to the antioxidant response element (ARE) to transcriptionally upregulate the expression cyto-protective genes to prevent oxidative stress damage. In addition, oxidative stress activates GSK3β, leading to phosphorylation and nuclear import of Src kinase, such as Fyn, Src, Yes, and Fgr, which phosphorylates Nrf2 (Tyr568) and promotes Nrf2 nuclear export and degradation [23, 24]. We previously found that sustained hyperglycemia and hyperlipidemia induced ROS increase, which resulted in the nuclear export of Nrf2 increase by GSK3β/Fyn signaling [25].

We recently reviewed the role of Nrf2 in stem cell state and function, focused on the role of Nrf2 in regulating redox and metabolic of stem cell [1]. The review demonstrated that the roles of Nrf2 in stem cell are unique and type- and lineage-specific. Several studies have confirmed that Nrf2 plays a critical role in stem cell survival and resistance to oxidative stress. Activating Nrf2 protects MSCs, HSCs, NSCs, and endothelial progenitor cells (EPCs) from apoptosis and death induced by oxidative stress through upregulating downstream anti-oxidative genes and growth factors. For example, Nrf2 overexpression protects BM MSCs from death induced by oxidative stress via upregulating HO-1 and SOD [26]. Furthermore, pharmacological activation of Nrf2 with Nrf2 activator, such as ginger oleoresin, could mitigate ionizing radiation-induced oxidative stress and DNA damage of human MSCs. Melatonin suppresses senescence of canine adipose MSCs via activating Nrf2 activation and inhibiting ER stress [27]. Elsewhere, Nrf2 is also crucial for HSCs survival and function under stress condition. Nrf2 deficiency leads to HSCs apoptosis increase and survival decrease under oxidative stress condition. Activating Nrf2 signaling, either by genetic methods or by pharmacological molecules, prevents radiation-induced HSCs injury by scavenging ROS [28, 29].

### 3.2.3 FoxOs Signal Pathway

Forkhead Box O (FoxO) proteins constitute an evolutionarily conserved family of transcription factors that play a critical role in the regulation of aging and longevity. In mammals, the FoxO family is composed of FoxO1, FoxO3a, FoxO4, and FoxO6, which are highly similar in structure, function, and regulation. FoxO transcription factors are important regulators of oxidative stress in mammals. On one hand, activated FoxOs can counteract oxidative stress via upregulating ROS-scavenging enzymes, such as MnSOD and catalase. On the other hand, ROS can regulate the expression and activity of FoxO proteins at multiple levels, including transcription and post-translation modification. For example, ROS indirectly regulates the

expression of FoxO through the upstream regulators of FoxO, such as p53, and miRNA. Furthermore, oxidative stress affects the activation and stabilization of FoxO through phosphorylation or deacetylation.

During the past decades, many evidences have shown that FoxO family members are critical mediators in regulating the self-renewal and maintaining homeostasis of NSCs/NPCs [30] and HSCs [31, 32]. The previous studies suggest that FoxOs play a central role in in keeping the long-term regenerative capability of the HSC compartment; deficiency of FoxOs in HSCs results in increase of ROS and decreased expression of ataxia telangiectasia mutated (ATM) that is key factor in coordinating stem cell cycling with ROS level. FoxO3 is a predominant FoxO isoform; the role FoxO3 in HSC is extensively explored. FoxO3 is a critical factor for protecting HSC from oxidative damage. Similarly, NSCs isolated from adult FoxO3(−/−) mice showed impairment of self-renewal and differentiation abilities with ROS accumulation [33]. The above findings suggest that FoxO plays a critical role in reducing ROS level and keeping redox balance. However, under oxidative stress condition, the expression and activity of FoxOs were altered by ROS. In EPCs, it was found that the expression of FoxO3a was markedly increased in EPCs treated with $H_2O_2$; increasing FoxO3a resulted in EPCs apoptosis possibly by transcriptional regulation of Bim [30]. FoxO transcription factors are essential for maintaining homeostasis of stem cell, but the mechanism of FoxOs in stem cell aging induced by oxidative stress needs further investigation.

### 3.2.4 Sirtuin Pathway

Sirtuins are a family of proteins with nicotinamide dinucleotide ($NAD^+$)-dependent deacylases activity, which play a pivotal role in delaying cellular senescence and extending the organismal life span via regulating various cellular processes. Lee et al. recently reviewed the roles of sirtuins on cellular senescence and life span extension [34]. In mammals, there are seven sirtuins (SIRT1–SIRT7) which are localized in different subcellular compartmentation with different enzymatic activity. SIRT1 prevents premature aging/cellular senescence by regulating FoxOs, p53 and p21 and molecules involved in DNA damage and repair. SIRT1 is a redox-sensitive protein; the level and activity of SIRT1 are regulated by oxidative stress mainly through posttranslational modification, such as phosphorylation, SUMOylation, S-glutathionylation, S-nitrosylation, and carbonylation [35]. In human lung epithelial cells, it was found that the oxidant significantly decreased the protein expression and activity of SIRT1 by carbonyl modifications [36]. Oxidative stress induces rat nucleus pulposus cell (NP) senescence and decreases the expression of SIRT1; specific activation of SIRT1 suppresses oxidative stress-induced senescence of NP cells by Akt-FoxO1 pathway [37]. The other group also found that inhibiting SIRT1 induced premature senescence of human endothelial cells, while overexpression of SIRT1 prevented hydrogen peroxide-induced premature senescence [38]. Besides SIRT1, SIRT6 also plays critical role in cell senescence and aging. SIRT6-deficient mice exhibited genomic instability and several

phenotypes of accelerated premature aging. SIRT6 could protect podocytes against apoptosis and inflammation by enhancing autophagy via inhibiting Notch signaling pathway [39].

Besides suppression mitotic cell senescence, sirtuins also regulate the senescence of stem cells. For example, the expression of SIRT1 in EPCs from old subjects was decreased; inhibition of SIRT1 in EPCs from young subjects resulted in marked increase of senescence [40]. Furthermore, cleaving SIRT1 with cathepsin leads to EPC premature senescence [41], while activating SIRT1 with MHY2233 can reduce replicative and oxidative stress-induced senescence in EPCs [42]. Similarly, SIRT1 declines with age and plays an essential role in maintaining MSC's long-term growth without undergoing cellular senescence; overexpression of SIRT1 in MSCs reduced osteogenic cell senescence and enhanced osteogenesis by deacetylating FoxO3a and inhibiting oxidative stress [43]. SIRT1 is also important for the maintenance of HSCs through ROS elimination, FoxO activation, and p53 inhibition [44]. Homma and colleagues have demonstrated that SIRT1 is important in maintaining phenotype and function of induced pluripotent stem (iPS)-derived vascular endothelial cells [45]. Similarly, the previous study demonstrated that SIRT6-deficient hMSCs showed functional decay and are more sensitive to the oxidative stress; further mechanism study uncovered that SIRT6 protects human MSCs against oxidative stress damage via coactiving Nrf2 signal pathway [46]. More recent study demonstrated that the expression of SIRT6 was decreased in BMSCs from the elderly and SIRT6 regulated senescence and osteogenesis of BMSCs via changing autophagy level partly through activating AKT-mTOR pathway [47]. SIRT6 also regulates HSC homeostasis and self-renewal capacity via epigenetic regulation of Wnt signaling [48] or repression NF-κB target genes [49]. The abovementioned findings indicated that SIRT1 or SIRT6 is believed to delay stem cell aging and senescence under the conditions of oxidative stress; activating SIRT1 or SIRT6 in stem cell would be a promising therapeutic strategy for chronic inflammatory diseases associated with aging. A lot of studies have showed that sirtuins activators, such as resveratrol, MHY2233, and melatonin, enhanced cell survival and reduced oxidative stress-mediated senescence in variety of kinds of stem cells [42, 50, 51].

### 3.2.5 Nuclear Factor Kappa B Pathway

Nuclear factor kappa B (NF-κB) is a family of globally expressed transcription factor involved in a series of cellular process, including proliferation, inflammation, differentiation, senescence, and apoptosis. The family of NF-κB comprises RelA/p65, RelB, c-Rel, p50/p100(NF-κB1), and p52/p100(NF-κB2). NF-κB pathway can be activated by two different pathways, the canonical and noncanonical pathways. The canonical pathway is induced by microbial products, stress factors, pro-inflammatory cytokines, and it depends on the activation of IκB kinase (IKK) complex, leading to phosphorylation and subsequent degradation of IκB molecules via the ubiquitin-proteasome system. Then, released NF-κB translocate into the nucleus to activate target gene expression. By contrast, the noncanonical pathway

is induced by B cell-activating factor (BAFF), lymphotoxin β (LTβ), CD40 ligand, CD27 ligand, human T-cell leukemia virus (HTLV), and Epstein-Barr virus (EBV) [39].

Cumulative evidences have indicated that the transcription activity of NF-κB can be directly elevated or inhibited by ROS in a context-dependent manner.

Study found that ROS increased phosphorylation of IκBα, which enables the nuclear translocation of NF-κB to enhance interleukin 8 (IL-8) secretion, and/or increased the stability of p53 protein, leading to cell aging [52]. Oxidative stress can activate NF-κB via triggering HSP90 to induce neural stem cell (NSC) death, while inhibition of HSP90 activity will attenuate the NF-κB/p65 activity under oxidative stress and promote NSCs survival [53]. Lin et al. reported that NF-κB activity was elevated in aged MSCs at basal condition or exposed to inflammatory stimuli; suppression of NF-κB activity in aged MSCs could potentially restore aging-associated bone loss or enhance bone-healing process [54]. Furthermore, the previous studies showed that NF-κB activation inhibits the myogenic differentiation of aged muscle-derived stem/progenitor cells (MDSPCs), while pharmacological or genetic inhibition of NF-κB activation improves myogenic differentiation and reduces oxidative stress-induced damage [55]. Aged HSCs also exhibit increased ground-stage NF-κB activity [56]; constitutive activation of NF-κB pathway leads to loss of HSCs quiescence and functions [57].

### 3.2.6 PI3K/Akt Signal Pathway

Phosphoinositide 3-kinase (PI3K)/Akt signaling pathway is one of most important prosurvival pathway in cells. Both PI3K and Akt kinases are responsive to cellular redox state. Akt possesses two conserved Cys residues (Cys297 and Cys311), which are susceptible to $H_2O_2$-mediated oxidation. $H_2O_2$ induces an intramolecular disulfide bond formation between Cys297 and Cys311of Akt, which leads to Akt dephosphorylation and Akt inactivation [58]. It is well known that PI3K/Akt pathway is a critical mediator in stem cell aging. Inhibition of PI3K-Akt pathway can accelerate cellular senescence in many kinds of stem cell, while activation of PI3K-Akt pathway could slow down the process of senescence. For example, doxorubicin induces MSCs aging via ROS accumulation, which associated with the activity of Akt decrease [59], while activating PI3K-Akt pathway by macrophage migration inhibitory factor (MIF) or high-density lipoprotein can rescue MSCs from oxidative stress-induced senescence or death [59, 60]. The main mechanism of these observations was attributed to the reduction in intracellular oxidative stress and prevents DNA damage. Furthermore, it was found that PI3K-Akt pathway inhibited senescence of human skin-derived precursors (hSKPs); enhancement of the activity of Akt in hSKP senescence could reduce expression of p53, p21, and p16 and mitigate hSKP senescence [61, 62]. Mammalian target of rapamycin (mTOR) is a direct target of Akt in regulating cell growth, metabolism, and autophagy. PI3K/Akt/mTOR signal pathway has been found to associate with cell senescence and aging. Increasing ROS generation correlates with PI3K/Akt/mTOR aberrant activation.

Inhibition of the PI3K/Akt/mTOR pathway prevented aging and prolonged life span, which is accompanied with suppression of ROS, iNOS, Cox-2, NF-κB, SASP, and p53 [63].

### 3.2.7 p38 MAPK Signal Pathway

The p38 mitogen-activated protein kinase (p38 MAPK), an important member of the MAPK family of signal transduction kinases, is activated in a sequential manner to regulate a variety of cellular process, such as self-renewal, proliferation, differentiation, senescence, and apoptosis. The signal pathway of p38 MAPK is known to be activated in variety of cells in response to oxidative stress. Considering the important role of oxidative stress in stem cell aging, it can be speculated that p38 may be an executor of oxidative stress-induced stem cell aging.

Many researches have showed that p38 signal pathway was activated during stem cell aging induced by elevated ROS. For instance, elevation of ROS levels actives p38 MAPK signal to limit the life span of HSCs; inhibition of p38 MAPK restored the defects of HSC repopulating capacity induced by ROS and extended the life span of HSCs, revealing that the ROS-p38 MAPK pathway contributes to HSC senescence and aging [64]. Connexin-43 deficiency resulted in HSC senescence; the mechanism indicated that connexin-43-deficient HSCs are unable to transfer ROS to the microenvironment, leading to cellular ROS accumulation, which activated p38MAPK/FoxO1 signal [65]. Similarly, thioredoxin-interacting protein also induced HSC aging via elevation of ROS to regulate p38 MAPK activity; inhibiting p38 MAPK activity can rejuvenate aged HSCs [66]. Interestingly, aged satellite cells (SCs) and bona fide muscle stem cells exhibit a cell-autonomous defect in self-renewal due to impaired response to FGF ligands and elevated p38 activity; inhibiting p38 MAPK activity can improve age-associated self-renewal defects [67]. The recent research demonstrated that p38 MAPK activation leads to decreases in the number and activity of ISC; targeting p38 MAPK prevents or rescues ISC aging [68]. Furthermore, there are many studies reporting that antioxidant compounds treatment improved the quantity and function of stem cells via downregulating the activation of p38 MAPK [69, 70].

The abovementioned research suggested that p38 MAPK signaling is a critical regulator in stem cell aging response to oxidative stress. This highlights the importance of uncovering the molecular mechanisms that link ROS production to activation of the p38 MAPK-mediated promotion of aging, longevity, and resistance to oxidative stress. Based on the previous studies on non-stem-cell models, it was known that oxidative stress activated p38 MAPK through two major pathways: one is that oxidative stress-mediated oxidation of apoptosis signal-regulating kinase (ASK1)-thioredoxin (Trx[SH]2) dissociates this complex, releasing activated ASK1-signalosome and leading p38MAPK activation [71–73]; the second is caused by activation of TGF-beta-activated kinase (TAK) 1 and TGF-beta-activated kinase 1-binding protein (TAB) 1 to form TGF-beta/TAK1/TAB1 complex, which activates p38 MAPK pathway either through canonical MAPKKKs or TGF-beta

directly through TAB1 by autophosphorylation [74–76]. However, whether oxidative stress activates p38 MAPK via the above pathways in stem cell aging is not clear.

### 3.2.8 microRNAs

microRNAs (miRNAs) are small single-stranded noncoding RNAs that regulate expression of target genes via modulating the translation or degradation of the targeted transcripts. A number of studies have revealed that miRNAs play a critical role in controlling oxidative stress-induced aging and cellular senescence [77–79]. For example, miR-34a and miR-570-3p are induced by oxidative stress and promote epithelial cell senescence by inhibiting sirtuin-1; inhibition of miR-34a or miR-570-3p can restore sirtuin-1 and suppress markers of cellular senescence [78, 79]. The more recent research reported oxidative stress downregulated expression of miR-20b-5p and miR-106a-5p to suppress the G1/S-phase transition of the cell cycle via promoting p21 expression and suppressing E2F1 in MSC [77]. miR-210 is also an important regulator in controlling stem cell function exposure to oxidative stress. Under oxidative stress condition, miR-210 expression was upregulated by ROS to increase the proliferation and migration of ASCs via targeting protein tyrosine phosphatase and nonreceptor type 2 expression [80]. Furthermore, it was also found that overexpression of miR-210 improved MSC survival under oxidative stress via antioxidation and c-Met pathway activation [81]. Similarly, Ham et al. report that let-7b protects MSCs from ROS damage via targeting caspase 3 [82]. These studies suggest that miRNAs that regulate ROS-induced stem cell are dependent on their distinct targets.

However, the mechanism underlying oxidative stress-regulated miRNAs expression remains to be demonstrated. The early studies showed that oxidative stress regulated aging-related miRNAs expression by either affecting the miRNA processing machinery or regulating the expression of certain specific miRNAs. Mori et al. found that multiple miRNAs were downregulated with age in large part due to a decrease of Dicer, which is one of the components of the miRNA processing machinery and required for synthesis of small interfering RNAs. In additional, they also found that oxidative stress resulted in Dicer expression decrease [83].

## 3.3 The Antioxidant Strategy of Stem Cell

Given the harmful effects of oxidative stress, stem cells possess several endogenous antioxidant stress strategies to manage ROS accumulation and adapt to oxidative stress. The strategies include resisting oxidative damage, removing excessive ROS, and inhibiting ROS production (Fig. 3.2). Under mild oxidative stress condition, stem cells resist or protect against oxidative damage by regulating antioxidant enzymes and apoptosis-associated gene expression. Conversely, sustained and intensive oxidative stress impairs stem cell self-renewal and accelerates senescence and

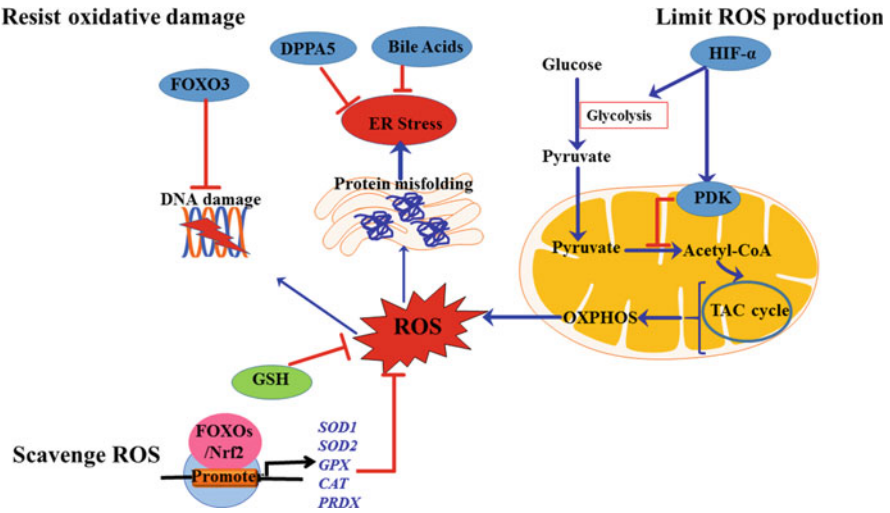


**Fig. 3.2** Strategies employed by stem cells to manage ROS accumulation and adapt to oxidative stress

apoptosis of stem cell. Thus, it is critical to understand the defensive anti-oxidative mechanism of stem cells for improving stem cell homeostasis and function.

Firstly, stem cells possess a protective mechanism to resist oxidative damage. For example, oxidative stress results in oxidative protein increase and impairs the protein folding abilities of endoplasmic reticulum (ER), which leads to misfolded proteins accumulation within the ER, resulting in ER stress and ER stress-associated ROS generation. Fetal liver HSCs can suppress oxidative protein aggregation and ER stress via bile acids [84]. Similarly, developmental pluripotency-associated 5 (Dppa5) is also reported to enhance long-term reconstitution of HSCs and improve the activity of HSCs via eliciting ER stress and apoptosis induced by oxidative stress [85]. Furthermore, HSCs can resist against oxidative damage of DNA through FoxO3-mediated upregulation of DNA repair components [31].

Secondly, stem cell prevents ROS accumulation and resists against ROS-induced damage through regulating the expression of endogenous antioxidant enzymes and antioxidants to scavenge excessive ROS. SOD is responsible for transforming $O_2 \cdot^-$ into $H_2O_2$, which is subsequently neutralized to $H_2O$ and O2 by catalases (CAT) or peroxidase, such as glutathione peroxidase (GPx) and peroxiredoxin (PRDX). The expression of antioxidant enzymes in stem cells was regulated by Nrf2 and FoxOs. Under oxidative stress condition, Nrf2 is released from Keap1 and translocated into nucleus, where it binds to ARE and upregulates antioxidant enzymes. In addition, glutathione, an important antioxidant molecule, plays a significant role in cellular ROS neutralization by scavenging ROS directly or serving as a substrate of glutathione peroxidases [86]. The previous studies found that high level of glutathione could maintain stem cell homeostasis and functions through inhibiting cell

senescence and preserving stemness and differentiation potential [87, 88]. Therefore, stem cells could manage ROS via ROS-scavenging enzymes and antioxidants.

Finally, stem cells could prevent ROS accumulation via inhibiting ROS generation. Mitochondria are primary sources of ROS in most cells, a consequence of its role in energy production via oxidative phosphorylation (OXPHOS). Stem cell possesses unique metabolic programs to limit ROS production. Stem cells preferentially utilize glycolysis rather than OXPHOS for adenosine-5′-triphosphate (ATP) production in a hypoxic microenvironment to maintain cell quiescence and function. HSCs generate ATP by anaerobic glycolysis through HIF-1α-mediated pyruvate dehydrogenase kinase (PDK) activation [89]. PDK suppress the activity of pyruvate dehydrogenase (PDH) and then block the entry of pyruvate for its catabolism into the tricarboxylic acid (TCA) cycle, thereby inhibiting influx of glycolytic metabolites into the mitochondria to reduce ROS generation. Lactate dehydrogenase A (LDHA) is also required for HSCs to facilitate glycolysis, inhibit OXPHOS, and reduce ROS production [90]. Other study demonstrates an unexpected role of FoxO3 in the maintenance of metabolic homeostasis in NPCs that counteracts oxidative stress and preserves their long-term proliferative potential. In additional, it was proved that FoxO3 is required for NPCs to regulate glucose metabolism via direct transcriptional activation of metabolic enzymes and decrease ROS accumulation [30]. Therefore, metabolism plays a critical role in maintaining redox homeostasis of stem cell.

## 3.4 Anti-oxidative Stress Strategy in Stem Cell Transplantation Therapy

Stem cell transplantation has emerged as a promising regenerative medicine therapy for a variety of degenerative disorders, such as ischemic stroke, heart attack, wound healing, and kidney failure. However, poor survival of transplanted cell is one of the major problems limiting the therapeutic efficacy. This is partly attributable to insufficient resistance of transplanted stem cell to severe oxidative stress at the injured sites. A recent development in combining the anti-oxidative stress strategy with stem cell therapy improves the efficiency of stem cell transplantation. Work from us and other groups showed that elevating the levels of antioxidants in stem cells with antioxidant molecules or anti-oxidative gene manipulation can significantly influence their survival and regeneration capacity. We summarized the recent researches about improving stem cell therapy efficacy with antioxidants in Table 3.1.

## 3.5 Conclusion

As stem cells are among the longest-living cells within an organism, stem cell aging may be a major driver of organismal aging. The consequences of stem aging include impaired self-renewal ability, loss of regenerative capacity, increased cell death, and depletion of stem cell pool. The process of stem cell aging is modulated by a range of intrinsic and extrinsic factors. Oxidative stress induces stem cell aging in many

**Table 3.1** Anti-oxidative stress strategy in stem cell transplantation therapy

| Disease | Model | Tansplanted stem cell | Antioxidant treatment | Benefits | Reference |
|---|---|---|---|---|---|
| Myocardial infarction | Lewis male rats with left anterior descending artery ligation | Bone marrow-derived MSCs | HO-1 overexpression | Enhanced anti-apoptotic and anti-oxidative capabilities of engrafted MSCs, and improves myocardial repair after ischemic injury | [91] |
| | SD male rats with left anterior descending coronary artery ligation | Bone marrow-derived MSCs | NAC | Enhances the adhesion of engrafted MSCs, and improves myocardial repair | [92] |
| | Male mdx mice with cardiotoxin injury | MDSCs | NAC | Increased the myogenic differentiation of MDSC, improved the survival of MDSCs and cardiac function, decreased scar tissue formation, and increased numbers of CD31 (+) endothelial cell structures | [93] |
| Stroke | Male SD rats with middle cerebral artery occlusion (MCAO) | Bone marrow-derived MSC | SOD3 overexpression | Reduced the infarct volume, improved neurological function, and promotes angiogenesis | [94] |
| | MCAO model in male SD rats | Bone marrow-derived MSC | Icariin | Increased MSCs cerebral homing and neuronal differentiation, reduced the infarct volume, improved neurological function, and promotes angiogenesis | [95] |
| | MCAO model in adult male C57BL/6 mice | NSCs | SOD1 overexpression | Increase the survival of grafted NSCs, enhances angiogenesis, reduced infarct size, and improved behavioral performance | [96] |
| | Intracerebral hemorrhage (ICH) model in C57BL/6 mice | NSCs | SOD1 overexpression | Protected the grafted NSCs, enhances neuroprotection, and facilitates behavioral recovery | [97] |

(continued)

**Table 3.1** (continued)

| Disease | Model | Tansplanted stem cell | Antioxidant treatment | Benefits | Reference |
|---|---|---|---|---|---|
| Hind limb ischemia diseases | Hind limb ischemia model in C57BL/6 mice | ADSCs | IGF-1C-modified hydrogel | Promoted survival and proangiogenic capacity of ADSCs, and ameliorated blood perfusion and muscle regeneration | [98] |
| | Hind limb ischemia in C57BL/6 mice | Bone marrow-derived MSC | Fucoidan | Enhanced MSC survival and proliferation in ischemic tissues, and improved functional recovery and limb salvage | [99] |
| Acute liver failure | Male NOD/SCID mice with Gal/LPS challenge | Human umbilical cord MSC | Edaravone | Improved hUCMSCs viability and morphology exposure to oxidative/inflammatory challenge, improves hepatic functions, and promotes host liver regeneration | [70] |
| Renal ischemia-reperfusion injury | Male SD rats with the release of bilateral renal pedicle clamps following occlusion | Bone marrow-derived MSC | Atorvastatin | Improved survival of implanted stem cells, and inhibited oxidative stress and inflammation in the ischemic kidney | [100] |
| | FVB mice with the release of renal pedicle clamps following occlusion | ADSCs | Chitosan-IGF-1C hydrogel | Enhanced the retention and survival of transplanted MSC, and protected MSC from oxidative stress | [101] |
| Acute interstitial cystitis | Male SD rats with cyclophosphamide | ADSCs | Melatonin | Increased the expressions of antioxidants, and attenuated inflammation | [102] |
| Wound healing | Full-thickness skin defect in STZ-induced diabetic mice | ADSC | HIF-1α overexpression | Promoted wound closure via enhancing angiogenic growth factor expression and suppressing ROS and 8-OHdG levels in ADSCs | [103] |

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| Xenotransplantation | Male NOD/SCID mice irradiated at a dose of 225 cGy | HSC | NAC | NAC-treated mice displayed a significant increase in human HSC engraftment and multilineage hematopoietic differentiation in the mice | [104] |

Abbreviations: *MSCs* mesenchymal stem cells, *MDSCs* muscle-derived stem cells, *NSCs* neural stem cells, *HSCs* hematopoietic stem cells, *NAC* N-acetylcysteine, *HO-1* heme oxygenase-1, *SOD* superoxide dismutase, *IGF-1* insulin-like growth factor-1

different signal pathways, which crosstalk with each other to form a complex regulation network. Recent studies have demonstrated the important role of oxidative stress in inducing stem cell aging and emphasized the possibility of maintaining intracellular homeostasis and improving transplantation efficiency with anti-oxidative stress strategy. Furthermore, the signal pathways of oxidative stress-induced stem cell aging have received increasing attention as targets to hinder stem cell aging. However, there are also some problems needed to be clarified in preventing stem cell aging via controlling oxidative stress in the future. Firstly, oxidative stress induces stem cell aging via regulating a complex signaling network, which may vary with different cell types. So, it is essential to define the detailed signaling network of different stem cell aging. Secondly, although emerging evidence has confirmed that antioxidant treatments improve stem cell survival and transplantation efficacy, there is lack of clinical trials to confirm which and whether antioxidants improve the stem cell therapy efficacy. Finally, different levels of ROS play pleiotropic roles in different stem cell populations; it requires the development and application of more advanced technologies and methods for ROS detection and molecular imaging to unveil the mechanisms underlying ROS-induced stem cell aging.

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