Oxidative Stress in Stem Cell Aging

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Abstract
Stem cell aging is a process in which stem cells progressively lose their ability to self-renew or differentiate, succumb to senescence or apoptosis, and eventually become functionally depleted. Unresolved oxidative stress and concomitant oxidative damages of cellular macromolecules including nucleic acids, proteins, lipids, and carbohydrates have been recognized to contribute to stem cell aging. Excessive production of reactive oxygen species and insufficient cellular antioxidant reserves compromise cell repair and metabolic homeostasis, which serves as a mechanistic switch for a variety of aging-related pathways. Understanding the molecular trigger, regulation, and outcomes of those signaling networks is critical for developing novel therapies for aging-related diseases by targeting stem cell aging. Here we explore the key features of stem cell aging biology, with an emphasis on the roles of oxidative stress in the aging process at the molecular level. As a concept of cytoprotection of stem cells in transplantation, we also discuss how systematic enhancement of endogenous antioxidant capacity before or during graft into tissues can potentially raise the efficacy of clinical therapy. Finally, future directions for elucidating the control of oxidative stress and developing preventive/curative strategies against stem cell aging are discussed.

Keywords
stem cell, oxidative stress, aging, signaling pathway, transplantation

Introduction
Stem cells are ontogenically diverse cell populations distributed in niches throughout the human body. They are unique in being pluripotent and endowed with a lifelong capacity for self-renewal. As stem cells can develop into a virtually infinite spectrum of cell types in organisms, prospects of their wide application in regenerative medicine, drug screening, and disease modeling have been suggested in the past decades. According to their origins, stem cells can be broadly divided into embryonic stem cells (ESCs) and adult stem cells (ASCs). Although ESCs are totipotent to form a brand new organism, studies on human ESCs are subject to ethical controversies, particularly in countries following the Christian faith.¹ To address this pitfall, ASCs have been extensively studied instead for applications in regenerative medicine,² on account of their apparent advantages in wide body distribution (e.g., bone marrow, peripheral blood, fat, cornea, retina, brain, skeletal muscle, dental pulp, liver, kidney, skin, gastrointestinal tract endothelium and pancreas), easy isolation, pluripotency, and immune-regulatory properties. Following transplantation, ASCs are capable of “sensing” locations of wound sites and migrating to those sites for tissue repair, through either direct reoccupying or paracrine actions to stimulate host organ regeneration.³

Aging is an essential physiological process in most cell types. When growth limits are reached, mammalian cells enter permanent cell cycle arrest, referred to as replicative senescence, mediated by internal factors such as telomere length, telomerase activity, and other aging-related genes.⁴,⁵ Another major aging mechanism, premature senescence, is affected by the cell microenvironment and can be elicited by stressful...
stimuli to initiate irreversible arrest of proliferation. For example, many intrinsic and extrinsic factors, such as changes in genetic structure and chromatin modifications, and nonoptimal culture conditions (e.g., nutrients, temperature, drugs, radiation, inflammation, mechanical stress, and oxidative stress), can induce replicative and premature senescence. Indeed, replicative senescence and premature senescence share many similarities, including a specific set of alternations in cell function, morphology, gene expression, and positive staining for senescence-associated β-galactosidase activity (SA-βgal). In contrast, genome-wide gene expression analysis show that cell aging due to different factors rarely overlap in the gene expression profile, which implies that the aging process is distinctly regulated by multiple signaling pathways.

**Aging Biology of Stem Cells**

Maintaining a balance between self-renewal and differentiation is extremely important for stem cell homeostasis, since excessive self-renewal favors carcinogenesis, while inordinate differentiation induces premature consumption. During replication, various stress stimuli promote stem cell aging through several signaling pathways, resulting in a series of cellular alterations, including cell morphological changes, compromised proliferation capacity, defective nutritional sensing, reduced genomic stability, telomere shortening, epigenetic alterations, impaired protein structures, mitochondrial dysfunction, dysregulated intercellular communication, and loss of cellular pluripotency. In a broad sense, major promoters of stem cell aging include accumulation of age-related toxin metabolites, DNA damage, protein oxidation, mitochondrial dysfunction, and stem cell depletion in aged tissues. Indeed, reactive oxygen species (ROS) generated from normal metabolism or extrinsic mediators are among the most important endogenous toxins and regulatory factors in stem cells. They are also the direct inductive factors for DNA, protein, and mitochondrial damage. Thus, from a general perspective, an emerging focus in stem cell aging biology is to understand the mechanisms that underpin the balance between oxidative stress and antioxidative defense in those cells. Concomitant with elevated ROS generation, an increased number of aged stem cells typically leads to a depletion of functional stem cell pools in aged tissues, through a combination of destabilizing events including perturbation of cell cycle activity, decline in self-renewal, improper differentiation, and stress-induced cell death (Fig. 1). Enhancing mitochondrial functions is a logical strategy for restoring aged stem cell function and tissue regeneration, since damaged mitochondria constitute a primary source of ROS, which can act as an inducer of apoptosis. For instance, in the past 100 years, caloric restriction (CR) has been employed to retard aging and extend the life span in diverse organisms including yeast and primates. It has been demonstrated that CR enhances the functions of various types of stem cells through mitochondria-related nutrient sensing and DNA damage pathways.

**Oxidative Stress–Evoked Pathways in Stem Cell Aging**

In simple terms, oxidative stress can be conceptualized as a suboptimal cellular status arising from deficits in the antioxidant reserve relative to ROS production. High levels of endogenous/exogenous active oxygen and excessive ROS are detrimental factors for the induction of cell aging and a large array of diseases, including cancer. ROS include free radicals such as superoxide (O2•−) and hydroxyl radicals (•OH) and nonpolar molecules such as hydrogen peroxide (H2O2), which are thought to be some of the principal ROS contributing to an oxidative stress environment. In general, high levels of ROS mediate oxidation of biological macromolecules such as DNA, lipids, and proteins, which consequently causes cellular damage through upsetting intracellular redox homeostasis, exhausting repair machineries, and inducing cell death via intrinsic and extrinsic apoptotic signaling. Emerging evidence suggests that oxidative stress plays a key role in stem cell aging induction and the progression of various diseases. As mentioned,
ROS-mediated damage to biological macromolecules can take the form of protein denaturation, lipid peroxidation, DNA modifications, and mitochondrial dysfunction, all of which ultimately promote cellular senescence. Other studies also emphasize that accumulation of damaged DNA and protein in stem cells may cause the senescence and loss of organ function. Given that mitochondria are a key hub of energy production and regulators of bioenergetics in stem cells, many studies provided corroborative evidence for a direct link between mitochondrial dysfunction and stem cell aging. Thus, understanding the complex signaling networks impacted by oxidative stress as well as interactions between those pathways and diseases is of paramount importance to establish the molecular basis for stem cell antistress capacities (Fig. 2). Here, we summarize several key pathways contributing to oxidative stress-related stem cell aging.

**Nuclear Factor-E2-Related Factor 2–Kelch-like ECH-Associated Protein 1 Pathway**

Nuclear factor (NF)-E2 nuclear factor erythroid 2-related factor 2 (Nrf2) is an important transcription factor that controls the cellular antioxidation machinery. In conjunction with Kelch-like ECH-associated protein 1 (Keap1) and antioxidant response element (ARE), which make up the Nrf2/Keap1/ARE pathway, the Nrf2 regulates the expression of a series of antioxidant enzymes in stem cells. In the absence of stress conditions, Keap1 is tightly bound to Nrf2 to suppress its activity, allowing a resting status. As oxidative stress sets in, redox-sensitive cysteine residues in the intervening region of Keap1 become oxidized, followed by dissociation of Nrf2 and Keap1 and Nrf2 nuclear translocation. Nrf2 further combines with transcription factor avian musculoaponeurotic fibrosarcoma (Maf), activating transcription factor 4, and Fos-related antigen proteins to form a heterodimer. ARE thus becomes activated to initiate transcription of a host of antioxidant genes so as to mobilize cellular detoxification.

A study conducted by Mohammadzadeh et al. proved that transient overexpression of Nrf2 protected mesenchymal stem cells (MSCs) against apoptosis triggered by hypoxia and oxidative stress via upregulation of superoxide dismutase (SOD) and heme oxygenase 1. Elsewhere, it was demonstrated that Nrf2 was crucial for several aspects of hematopoietic stem cell (HSC) homeostasis. Specifically, deficiency of Nrf2 induced cell-intrinsic hyperproliferation and impaired the migration and retention of HSCs in their bone marrow niche, partly through direct interaction between Nrf2 and C-X-C chemokine receptor type 4 (CXCR4). Subsequent studies of Keap1-knockout mice further revealed a critical role of Nrf2 in cell fate determination and cellular ROS regulation of HSCs and human airway basal stem cells. A very recent study identified a coordinated regulatory system of a cilium-autophagy-Nrf2 control axis and cell cycle progression that directs human ESCs toward neuroectoderm, which was the first decision during ESC differentiation. Antioxidant treatment (e.g., edaravone and N-acetylcysteine [NAC]) has been shown to relieve oxidative stress–suppressed Nrf2 activity in MSCs (e.g., from umbilical cord and muscle), leading to enhanced antistress capacities against exogenous toxin challenge and improved transplantation efficacy in animal models of acute liver failure.

### Wnt/β-Catenin Pathway

When the Wnt ligand binds to cell membrane co-receptors’ low-density lipoprotein receptor-related proteins 5/6 (LRP5/6) or Frizzled receptors, it either activates disheveled protein in the cytoplasm to directly suppress glycogen synthase kinase 3β (GSK-3β) activity or induces the accumulation of β-catenin in the cytoplasm. When a certain cytoplasmic threshold level is reached, β-catenin translocates itself into the nucleus to act as a transcriptional coactivator of transcription factors that belong to the family of T-cell factor/lymphoid-enhancer factor. Numerous studies supported an indispensable role of the Wnt/β-catenin pathway in cell proliferation, differentiation, apoptosis, cell position decision, and carcinogenesis.

It has been suggested that the Wnt/β-catenin pathway is implicated in the process of stem cell aging brought on by microenvironmental changes. For example, several studies found that sustained Wnt/β-catenin exposure was an important factor to promote ASC aging in both in vitro and in vivo models. When incubated with old rat serum, rat MSCs exhibited typical senescence phenotypes including reduced proliferation and increased SA-βgal and ROS production. This was partly a result of the activation of the DNA damage response and the p53/p21 pathway evoked by the Wnt/β-catenin pathway. A recent seminal work by Florian et al. confirmed that during HSC aging, elevated levels of Wnt5a caused an unexpected shift from canonical to noncanonical signaling.
Wnt signaling. Subsequent studies with transgenic animals found that Wnt5a treatment of young HSCs exhibited aging-associated phenotypes, while haplo-insufficiency of Wnt5a attenuated HSC aging. It was reported that chronic ethanol administration suppressed osteoblastogenesis and enhanced adipogenesis of MSCs, leading to evident reduction in bone mineral density. Treatment with antioxidants (e.g., NAC) significantly blocked bone loss and rebalanced stem cell differentiation directly through the recovery of Wnt/β-catenin signaling inhibited by ethanol.

**p53 Pathway**

p53 (tumor protein p53, or tp53) was recognized as one of the most important genes with cellular tumorigenesis. In response to myriad stressors, including DNA damage and oxidative stress, p53 is activated as a transcription regulator, leading to a quick accumulation of p53 in stressed cells. In addition, p53 exerts its influence over a variety of key protein pathways through posttranslational modifications including protein phosphorylation, acetylation, methylation, and ubiquitination. Thus, p53 serves as a “genome guardian” to monitor the integrity of the genome and maintain cell homeostasis.

It has been suggested that oxidative stress elicits a specific p53 transcriptional response, which is mediated by p44/p53 and p66 to control cellular senescence and aging. Studies have also found that oxidative stress could enhance the protein acetylation levels of p53 and promote cell aging, implicating the NAD⁺-dependent deacetylase enzyme sirtuin 1 (SIRT1). However, oxidative stress environments do not always induce cell aging, as evidence exists that when cells are maintained at a low level of oxidative stress, p53 primarily induces the expression of antioxidant genes to prevent cell death. By contrast, high levels of p53 could accelerate the generation of ROS and induce cell death under severe cellular stress. In stem cells, in response to telomere attrition and oncogenic stimuli, activation of p53 depletes HSCs. Besides mTOR and Wnt, p53-induced phosphatase 1 (Wip1) was also shown to regulate HSC aging through direct p53 actions, since Wip1-knockout mice exhibited multifaceted phenotypes of HSC aging while deletion of p53 rescued the condition. However, the precise role of p53 in stem cell aging is complex as others have found that blocking of the PI3K/Akt/mTOR pathway prevented aging phenotypes and enhanced proliferative capacity of MSCs. Reduction in intracellular oxidative stress, prevention of DNA damage, and induction of pluripotency gene expression (e.g., Nanog and octamer-binding transcription factor 4) were thought to be the main mechanisms underlying the observations.

**Nuclear Factor-Kappa B Pathway**

Nuclear factor-kappa B (NF-κB) is a master transcriptional regulator of immune response and cell death. It is well-known that oxidative stress triggers inflammatory cascades that are primarily mediated by NF-κB. Study found that ROS activated inhibitors of NF-κB (IkBα) ubiquitination, NF-κB translocation, the stimulation of interleukin 8 (IL-8) expression, and/or increase of p53 protein stability, leading to cell aging intervention. This finding was further confirmed in induced pluripotent stem cells (iPSCs): NF-κB was repressed during cell reprogramming toward their pluripotent state while hyperactivation of aging-associated NF-κB inhibits iPSC generation via eliciting the
reprogramming repressor DOT1-like histone H3K79 methyltransferase (DOTI).

Furthermore, p65 isoform of NF-κB was activated and accumulated in aged HSCs, most likely increasing the expression of P-selectin and reflecting a time-dependent increase in inflammation. IGF-1, mTOR, SIRT1, and p53 are reported to be the upstream signaling regulator of the NF-κB pathway during aging. Attenuation of NF-κB activity (primarily p65) by heat shock protein 90 (HSP90) inhibitor, NAC, myoblast determination protein (MyoD), and NF-κB small molecule inhibitor was reported to reduce cellular oxidative stress, alleviate cell death, and enhance stemness in various stem cell types.

**Mitogen-Activated Protein Kinase Signaling Pathway**

Mitogen-activated protein kinase (MAPK) is a family of serine/threonine protein kinases that are widely distributed in mammals and mainly includes extracellular signal-regulated kinase 1/2 (ERK1/2), c-JUN N-terminal kinase (JNK), p38, and ERK5 members. MAPK has been identified as a major regulator in cell growth, differentiation, stress environment, cell death, and inflammatory response. This pathway can be activated by various extracellular stimuli such as physical cues, inflammatory cytokines, growth factors, and bacterial components.

The roles of MAPK in cell aging have been investigated in a number of studies. For example, when compared with young mice, aged mouse livers exhibited decreased ERK1/2 level but increased JNK1/2 and p38 MAPK levels. However, data from rat aorta and human skeletal muscle were distinct, indicating that MAPK is affected by aging in a tissue-specific manner. It was also found that signaling pathways evoked by oxidative stress were stimulus source dependent, since endogenous H_{2}O_{2} produced from hypoxic stress induced ERK activation, while exogenous H_{2}O_{2} primarily induced p38 MAPK activation. In HSCs, p38 MAPK is activated by ROS to augment the expression of p16 and p19 alternate reading frame (ARF), thus limiting the self-renewal capacity of HSCs as well as the life span of mice. Interestingly, in aged skeletal muscle, a stem cell autonomous loss of self-renewal potential was investigated with alterations in p38 MAPK and fibroblast growth factor receptor 1 signaling. Pharmacological manipulation of p38 MAPK significantly alleviated age-associated stem cell self-renewal defects. Furthermore, evidence exists for crosstalk between the MAPK and Nrf2 pathways, since oxidative stress activates the MAPK-Nrf2-ARE axis to regulate phase II enzyme (e.g., SOD and glutathione S-transferase) expression and activity, which consequently modulates cell growth and aging. By treating stem cells with antioxidant compounds (e.g., NAC, edaravone, or zeaxanthin dipalmitate), the oxidative stress–evoked activation of MAPK pathway components (e.g., p38 MAPK and ERK) was significantly suppressed, which facilitates ROS detoxification and attenuation of cell injury. However, it seems that other key proteins, particularly those downstream of MAPKs, also play substantial roles in the antioxidative defense of stem cells against injury and aging.

**Sirtuin Pathway**

Sirtuins are a class of proteins with NAD-dependent deacetylase activity. They are functionally significant in regulating stem cell proliferation, death, differentiation, and aging, particularly in the contexts of oxidative stress. SIRT1 and adenosine monophosphate-activated protein kinase (AMPK) were reported to coordinate aging-impaired cell growth, differentiation, and mitochondrial functions of MSCs. A recent study found that miR-34a mediates this process by acting as pro-apoptotic and pro-senescence factors in MSCs via direct targeting of SIRT1. Inhibition of miR-34a resulted in improved viability and antistress functions of MSCs in an in vitro model of hypoxia and serum starvation. In addition, the osteogenic and adipogenic differentiation of tendon or dental pulp stem cells were also controlled by SIRT1, studies on the roles of SIRT2 in stem cell biology are scarce. Unlike SIRT1, studies on the roles of SIRT2 in embryonic body differentiation of mouse ESCs. Knockdown of SIRT2 shifted ectoderm to mesoderm/endoderm differentiation through the GSK-3β activation. As a regulator of mitochondrial homeostasis and oxidative stress, SIRT3 is dispensable for young HSC functional maintenance but becomes essential at an old age. Controlling mitochondrial plasticity and SIRT3 expression is a promising strategy to reverse age-related degenerations. Other studies have also suggested that decreased SIRT3 and NAD^{+} levels in aged MSCs and somatic cells increased cell vulnerability to oxidative stress and senescence. The roles of SIRT6 in stem cell oxidative stress and aging have been intensively investigated over the past decade. It is one of the few known genes that can modulate both longevity and progeria, since over-expression of SIRT6 led to enhanced longevity and SIRT6-deficiency-induced progeroid phenotypes. A recent study illustrated a protective role of SIRT6 against oxidative stress–induced MSC injury, in which SIRT6 coactivates Nrf2 and RNA polymerase II. Data from an HSC homeostatic study highlighted the importance of epigenetic regulation of Wnt signaling for ASC homeostasis and self-renewal capacity. Furthermore, the NF-κB pathway was exhibited to be quite crucial in SIRT6-mediated stem cell differentiation and senescence.

An appropriate treatment using the antioxidant NAC has been found to postpone the aging process of mouse oocytes partly through the upregulation of both SIRT1 and SIRT2. In a mouse model of laminopathy-based progeria, a potent antioxidant agent from grape seed, resveratrol, reportedly rescued SIRT1-dependent ASC decline and extended the life span of mice. Lamin A was identified as a direct activator of SIRT1 to induce deacetylase inactivation. In hESC-derived mesenchymal progenitors, resveratrol promoted cell...
osteoogenesis but inhibited adipogenesis via the SIRT1/ forkhead box O3a (FoxO3a) axis. Although the direct involvement of oxidative stress was not investigated in details, it was speculated that major ROS-related pathways were preferentially modulated to enhance the therapeutic potential of antioxidants.

**Autophagic Pathway**

Since accumulation of damaged proteins and organelles is closely associated with aging and senescence, emerging evidence suggests that deficiency of autophagy–lysosomal activity underpins cell aging. In HSCs, deletion of autophagy-related gene 7 (Atg7) induced mitochondrial dysfunction, DNA damage, and oxidative stress. Warr et al. further proved that during HSC aging, FoxO3A-induced autophagy was indispensable for metabolic stress protection. Notably, mTOR signaling in intestinal stem cells was shown to regulate the self-renewal and intestinal niche function maintenance. To preserve cell integrity, young quiescent muscle stem cells often use autophagy to guarantee cellular homeostasis and as a protein quality control strategy. However, autophagic regulation is significantly impaired in aged stem cells, which negatively affects the ability of the cells to provide nutrients for the transition from quiescence to activation or maintain stemness by preventing senescence. Although not well-defined, the mechanistic link between autophagy and other key cellular events (e.g., inflammation and apoptosis) should also be quite important for stem cell aging.

Numerous lines of evidence have converged on the idea that autophagy could be a therapeutic target of antioxidant agents. For example, human umbilical cord MSCs with irradiation injury were protected by starvation- or rapamycin-induced autophagy through decreasing ROS production. Treatment with NAC partially mimicked this process. Moreover, H$_2$O$_2$-induced hMSC mitoptosis, necroptosis, and apoptosis were attenuated by a synergistic protection of NAC and ascorbic acid 2-phosphate. The cellular antioxidation machinery encompasses several key pathways. Firstly, through the regulation of endogenous antioxidant enzymes (e.g., SOD, catalase, glutathione peroxidase) and other nonenzymatic molecules (e.g., ergothioneine, vitamin C, microelement), cells efficiently remove excessive oxidants and protect important organelles from ROS-induced damage. Secondly, with the facilitation of antioxidant molecules, cells are able to inhibit cellular senescence and apoptosis by regulating the expression of apoptosis-related genes such as Bcl-2, Bax, p53, and inhibitors of apoptosis proteins. Essentially, the antioxidant

**MicroRNAs**

MicroRNAs (miRNAs) are endogenous nontranscriptional small RNA of 20–24 nucleotides in length. They function as crucial posttranscriptional regulators in diverse processes including gene expression, protein synthesis, and even cell differentiation and apoptosis. In particular, an essential role of miRNAs in ESC differentiation has been recognized. For example, loss of miRNA-processing enzyme Dicer caused ESC differentiation defects and the death of mouse embryos during early development. The pathological association between oxidative stress and microRNA-related pathways has been established by several recent studies. In adipose tissue-derived stem cells, ROS from hypoxia, antimycin, rotenone, or platelet-derived growth factor (PDGF) upregulated miR-210 expression and increased stem cell proliferation/migration via protein tyrosine phosphatase, nonreceptor type 2. Our study found that regulation of miR-210 expression by zeaxanthin dipalmitate was important for the maintenance of cellular detoxification capacity against oxidative stress and the enhancement of transplantation efficacy in an acute liver failure model. It was subsequently found that overexpression of miR-210 could enhance MSC antioxidant ability and survival after ROS challenge through the activation of c-mesenchymal–epithelial transition. In addition, it was found that direct binding of miR-141-3p to zinc metalloproteinase STE24 transcripts was responsible for pre lamin A accumulation in the nuclear envelope of human MSCs, which led to cellular senescence. Maturation of lethal 7 was demonstrated to bind high-mobility group AT-hook 2 to regulate the inhibitor of cyclin-dependent kinase type 4/Arf expression, which was crucial for the decrease in the ability of neural stem cell self-renewal.

**The Antioxidant Response Pathways in Stem Cells**

Cellular redox status significantly influences stem cell homeostasis. The existence of oxidative stress frequently demands an adaptive response from the endogenous antioxidant stress machinery, which in turn significantly modulates the level of oxidative stress. On close inspection, stem cells may respond differentially to different levels of oxidative stress. Under a mild stress condition, cells mainly regulate apoptosis-related gene expression, antioxidant enzyme activity, and defensive transduction pathways to fulfill antioxidative needs. In contrast, sustained and intense oxidative stress dampens stem cell proliferation and promotes premature aging, apoptosis, and even tumor formation. Therefore, understanding the defensive antioxidative responses of stem cells has both theoretical and practical significance for improving stem cell homeostasis and clinical transplantation.

The cellular antioxidation machinery encompasses several key pathways. Firstly, through the regulation of endogenous antioxidant enzymes (e.g., SOD, catalase, glutathione peroxidase) and other nonenzymatic molecules (e.g., ergothioneine, vitamin C, microelement), cells efficiently remove excessive oxidants and protect important organelles from ROS-induced damage. Secondly, with the facilitation of antioxidant molecules, cells are able to inhibit cellular senescence and apoptosis by regulating the expression of apoptosis-related genes such as Bcl-2, Bad, p53, and inhibitors of apoptosis proteins. Essentially, the antioxidant
Acute interstitial myocardial failure. Healthy mice for renin ischemia–reperfusion injury. Acute liver failure. Acute interstitial cystitis. Renal ischemia–reperfusion injury. Male mdx mice with cardiotonin injury. Male SD rats with left anterior descending coronary artery ligation. Male NOD/SCID mice with gal/LPS challenge. Male SD rats with cyclophosphamide. Male SD rats with the release of bilateral renal pedicle clamps following occlusion. FVB mice. Cultured cells with FeCl₂ (100 μM) followed by H₂O₂ (50 μM).

**Table 1. Summary of Studies Using Antioxidants to Improve Efficacy of Stem Cell Therapy.**

| Disease                              | Model                                      | Implanted Stem Cell                      | Antioxidant Treatment | Improvement                                      |
|--------------------------------------|--------------------------------------------|------------------------------------------|-----------------------|-------------------------------------------------|
| Myocardial infarction²³              | Male mdx mice with cardiotonin injury      | Muscle-derived stem cells (1 x 10⁶ cells) | N-acetylcysteine      | Improves cardiac functions and decreases scar tissue formation |
| Myocardial infarction¹²⁰             | Male SD rats with left anterior descending coronary artery ligation | Mesenchymal stem cells (2 x 10⁶ cells) | N-acetylcysteine      | Transplantation efficacy was higher in implanted stem cells from young donor rats than that of old donor rats. |
| Acute liver failure³⁶               | Male NOD/SCID mice with Gal/LPS challenge | Umbilical cord mesenchymal stem cells, (2 x 10⁶ cells) | Edaravone            | Increases implanted stem cell number, improves hepatic functions, and promotes host liver regeneration |
| Acute interstitial cystitis¹²¹       | Male SD rats with cyclophosphamide         | Adipose-derived mesenchymal stem cells (1.2 x 10⁶ cells) | Melatonin            | Increased stem cell-mediated AIC amelioration through tissue inflammation and oxidative stress regulation |
| Renal ischemia–reperfusion injury¹²² | Male SD rats with the release of bilateral renal pedicle clamps following occlusion | Bone marrow mesenchymal stem cells (1 x 10⁶ cells) | Atorvastatin         | Improves survival of implanted stem cells, enhances injury amelioration outcomes |
| Healthy mice for transplantation efficacy test¹²³ | FVB mice | Adipose-derived mesenchymal stem cells (7 x 10⁵ cells) | Over-expression of endogenous SOD2 expression | Improves survival of grafted cells in early stages after implant. |
| In vitro OH·-radical-induced cell damage¹²⁴ | Cultured cells with FeCl₂ (100 μM) followed by H₂O₂ (50 μM) | Bone marrow mesenchymal stem cells | Dihydromyricetin     | Improves cell viability through scavenging O₂⁻ and DPPH radical |

Abbreviations: SOD2, superoxide dismutase 2; AIC, acute interstitial cystitis; DPPH, 1,1-diphenyl-2-picrylhydrazyl; FVB, B strain of the Friend murine leukemia virus; Gal/LPS, D-galactosamine with lipopolysaccharide; NOD/SCID, non-obese diabetic/severe combined immune deficiency; SOD2, superoxide dismutase 2.

effect in stem cells does not take place in a single approach but through the multiple signaling pathways.

### Controlling Oxidative Stress as a Therapeutic Strategy in Regenerative Medicine

Although stem cell transplantation has been shown to improve clinical outcomes in a number of diseases, poor cell survival following transplantation has limited its efficacy. This is mainly attributable to the severe inflammation and oxidative stress that negatively impact the microenvironment at the sites of injury. Some animal studies and clinical trials have also suggested that oxidative stress is negatively associated with stem cell transplantation efficacy. Accordingly, it has been posited that through systematic intervention that enhances resistance to oxidative stress, the therapeutic efficacy of stem cells could significantly improve. Indeed, resistance to stress are regarded as a cardinal characteristic among stem cells. A growing body of evidence has revealed that oxidative stress tolerance was mainly due to intrinsically high expression and activity of antioxidant enzymes. Meanwhile, it has also been reported that treatment with antioxidants such as NAC and edaravone with stem cells allows significantly improved survival and tissue repair capacity. In summary, stem cell aging not only diminishes their structural integrity and ability to repair tissue but is also detrimental to stem cell transplantation efficacy. Clearly, a better understanding of the complex regulatory networks controlling stem cell health and aging is critical for delaying the senescence and improving the clinical outcomes of stem cell transplantation. Recent studies using antioxidants to improve stem cell therapy efficacy are summarized in Table 1.

### Conclusion and Perspectives

The consequences of stem cell aging can be both subtle and overt, ranging from impairment of cellular homeostasis, vulnerability to cellular damage, and loss of regenerative function, to increased cell death. This process is modulated by many different intrinsic and extrinsic pathways, whose signaling is more complex due to complex cross talk. The importance of oxidative stress in inducing stem cell aging has been amply illustrated by recent studies, which emphasized the possibility of maintaining cellular homeostasis and enhancing transplantation efficacy through regulation of the endogenous antioxidation machinery. In addition, pathways for DNA damage repair, protein translation/stability, maintenance of mitochondrial function, and stem cell pool replenishment in aged tissues are receiving increasing attention in recent years as targets to prevent stem cell aging. However, several major problems need to be addressed in the context of oxidative stress control and stem cell aging retardation in the near future. Specifically:
Interdependence of aging pathways means that it would be difficult to ascertain the pathway that is responsible for certain aging phenotypes or clinical conditions. In most cases, some signals have a greater influence over an aging event. For example, Nrf2 is a general regulatory transcriptional factor for regulation of antioxidant genes and NF-κB is the master regulator of cellular inflammation and apoptosis in aged or damaged stem cells. Defining the detailed signaling network is critical for the study of stem cell aging biology.

Although many studies supported the effectiveness of antioxidant treatments (e.g., NAC) in improving stem cell antistress ability, antiaging processes, and transplantation efficacy, it remains unclear how those treatments affect age-dependent deficits of stem cells. In addition, further identification and characterization of the direct binding partners or molecular targets of specific antioxidants is required.

It is clear that uncontrolled ROS is the major inducer of stem cell aging. However, the pleiotropic roles of ROS in different stem cell populations during distinct phases of aging need to be established. Moreover, whether low levels of endogenous ROS are beneficial to stem cell homeostasis is also a cogent question to answer.

Transplantation of functional stem cells has been well explored recently as a method to replenish aged or damaged cells in the context of degenerative diseases, such as neurological disorders. However, for the sake of clinical therapy, simple replacement of aged stem cells with young ones is unlikely to be sufficient since a variety of nonautonomous signals contribute to stem cell aging (e.g., extracellular stress signals). Therefore, potentiating the paracrine effects of implanted stem cells and safeguarding the residual aged stem cell functions seem to be practical therapeutic strategies.

Genetic manipulation of stem cells (e.g., overexpression of antioxidant gene by lentiviruses) has been shown to enhance the antistress capacity of stem cells against exogenous stressors. This strategy should be handled very carefully since uncontrolled alteration of genomic DNA may induce carcinogenesis. Furthermore, prevention of telomere attrition by upregulating the telomeric pathway is capable of rescuing lost functions of aged stem cells, but this pathway can also be exploited by cancer (stem) cells to overcome replicative senescence. Transient overexpression of key antioxidant enzymes (except a few examples) did not significantly influence the life span.

In addition, there has been a lack of clinical trials to validate any correlation between the levels of antioxidants and the human life span. The complexity of the issues may be understood in terms of the sources, nature, subcellular localization and dynamics of cellular oxidative species formed, and which antioxidants could potentially neutralize an oxidant with specificity. Future investigation in this area would demand the development and application of even more sophisticated technologies and methods for ROS detection and molecular imaging so as to unravel the chemical and biological aspects of mechanisms underlying stem cell aging. Overall, initiatives to understand how antioxidant levels of stem cells can be controlled before or during transplantation promise to provide a viable approach for developing translational applications in regenerative medicine.

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References
1. Ilic D, Ogilvie C. Human embryonic stem cells-what have we done? What are we doing? Where are we going? Stem Cells. 2016;35(1):17–25.
2. Xin T, Greco V, Myung P. Hardwiring stem cell communication through tissue structure. Cell. 2016;164(6):1212–1225.
3. Wagers AJ, Weissman IL. Plasticity of adult stem cells. Cell. 2004;116(5):639–648.
4. Ocampo A, Reddy P, Izpisua Belmonte JC. Anti-aging strategies based on cellular reprogramming. Trends Mol Med. 2016;22(8):725–738.
5. Wege H, Chui MS, Le HT, Strom SC, Zern MA. In vitro expansion of human hepatocytes is restricted by telomere-dependent replicative aging. Cell Transplant. 2003;12(8):897–906.
6. Fehrer C, Lepperdinger G. Mesenchymal stem cell aging. Exp Gerontol. 2005;40(12):926–930.
7. Rohani L, Johnson AA, Arnold A, Stolzing A. The aging signature: a hallmark of induced pluripotent stem cells? Aging Cell. 2014;13(1):2–7.
8. Erusalisky JD, Skene C. Mechanisms of endothelial senescence. Exp Physiol. 2009;94(3):299–304.
9. Schwarze SR, DePrimo SE, Grabert LM, Fu VX, Brooks JD, Jarrard DF. Novel pathways associated with bypassing cellular
senescence in human prostate epithelial cells. J Biol Chem. 2002;277(17):14877–14883.
10. de Magalhaes JP, Chainiaux F, de Longueville F, Mainfroid V, Migeot V, Marcq L, Remacle J, Salmon M, Toussaint O. Gene expression and regulation in h2o2-induced premature senescence of human foreskin fibroblasts expressing or not telomerase. Exp Gerontol. 2004;39(9):1379–1389.
11. Doe CQ. Neural stem cells: balancing self-renewal with differentiation. Development. 2008;135(9):1575–1587.
12. Signer RA, Morrison SJ. Mechanisms that regulate stem cell aging and life span. Cell Stem Cell. 2013;12(2):152–165.
13. Ho JH, Chen YF, Ma WH, Tseng TC, Chen MH, Lee OK. Cell contact accelerates replicative senescence of human mesenchymal stem cells independent of telomere shortening and p53 activation: roles of ras and oxidative stress. Cell Transplant. 2011;20(8):1209–1220.
14. Oh J, Lee YD, Wagers AJ. Stem cell aging: mechanisms, regulators and therapeutic opportunities. Nat Med. 2014;20(8):870–880.
15. Pervaiz S, Tanega R, Ghaffari S. Oxidative stress regulation of stem and progenitor cells. Antioxid Redox Signal. 2009;11(11):2777–2789.
16. Sohal RS, Agarwal S, Candas M, Forster MJ, Lal H. Effect of age and caloric restriction on DNA oxidative damage in different tissues of c57bl/6 mice. Mech Ageing Dev. 1994;76(2–3):215–224.
17. Gredilla R, Sanz A, Lopez-Torres M, Barja G. Caloric restriction decreases mitochondrial free radical generation at complex i and lowers oxidative damage to mitochondrial DNA in the rat heart. FASEB J. 2001;15(9):1589–1591.
18. Schulz TJ, Zarse K, Voigt A, Urban N, Birringer M, Ristow M. Glucose restriction extends caenorhabditis elegans life span by inducing mitochondrial respiration and increasing oxidative stress. Cell Metab. 2007;6(4):280–293.
19. Pirinen E, Canto C, Jo YS, Morato L, Zhang H, Menzies KJ, Williams EG, Mouchiroul L, Moullan N, Hagberg C, et al. Pharmacological inhibition of poly(adenosine diphosphate-ribose) polymerases improves fitness and mitochondrial function in skeletal muscle. Cell Metab. 2014;19(6):1034–1041.
20. Zhang H, Ryu D, Wu Y, Gariani K, Wang X, Luan P, D’Amico D, Ropelle ER, Lutolf MP, Aebersold R, et al. Nad(+) repletion improves mitochondrial and stem cell function and enhances life span in mice. Science. 2016;352(6292):1436–1443.
21. Xu J, Huang Z, Lin L, Fu M, Gao Y, Shen Y, Zou Y, Sun A, Qian J, Ge J. Mir-210 over-expression enhances mesenchymal stem cell survival in an oxidative stress environment through antioxidation and c-Met pathway activation. Sci China Life Sci. 2014;57(10):989–997.
22. Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. Curr Biol. 2014;24(10):R453–R462.
23. Abe M, Takiguchi Y, Ichimaru S, Tsuchiya K, Wada K. Comparison of the protective effect of N-acetylcysteine by different treatments on rat myocardial ischemia-reperfusion injury. J Pharmacol Sci. 2008;106(4):571–577.
24. Pollina EA, Brunet A. Epigenetic regulation of aging stem cells. Oncogene. 2011;30(28):3105–3126.
25. Bucciantini M, Giannoni E, Chiti F, Baroni F, Formigli L, Zurdo J, Taddei N, Ramponi G, Dobson CM, Stefani M. Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. Nature. 2002;416(6880):507–511.
26. Mandal PK, Blanpain C, Rossi DJ. DNA damage response in adult stem cells: pathways and consequences. Nat Rev Mol Cell Biol. 2011;12(3):198–202.
27. Bratic A, Larsson NG. The role of mitochondria in aging. J Clin Invest. 2013;123(3):951–957.
28. Ahlvqvist KJ, Suomalainen A, Hamalainen RH. Stem cells, mitochondria and aging. Biochim Biophys Acta. 2015;1847(11):1380–1386.
29. Min-Wen JC, Jun-Hao ET, Shyh-Chang N. Stem cell mitochondria during aging. Semin Cell Dev Biol. 2016;52:110–118.
30. Lu MC, Ji JA, Jiang ZY, You QD. The keap1-nrf2-are pathway as a potential preventive and therapeutic target: an update. Med Res Rev. 2016;36(5):924–963.
31. Mohammadzadeh M, Halabian R, Gharehbaghian A, Amirizadeh N, Jahanian-Najafabadi A, Roushandeh AM, Roudkaren MH. Nrf-2 overexpression in mesenchymal stem cells reduces oxidative stress-induced apoptosis and cytotoxicity. Cell Stress Chaperones. 2012;17(5):553–565.
32. Tsai JJ, Dudakov JA, Takahashi K, Shieh JH, Velardi E, Holland AM, Singer NV, West ML, Smith OM, Young LF, et al. Nrf2 regulates haematopoietic stem cell function. Nat Cell Biol. 2013;15(3):309–316.
33. Murakami S, Shimizu R, Romeo PH, Yamamoto M, Motohashi H. Keap1-nrf2 system regulates cell fate determination of hematopoietic stem cells. Genes Cells. 2014;19(3):239–253.
34. Paul MK, Bishi T, Darmawan DO, Chiou R, Ha VL, Wallace WD, Chon AT, Hegab AE, Grogan T, Elashoff DA, et al. Dynamic changes in intracellular os levels regulate airway basal stem cell homeostasis through nrf2-dependent notch signaling. Cell Stem Cell. 2014;15(2):199–214.
35. Jang J, Wang Y, Lalli MA, Guzman E, Godshalk SE, Zhou H, Kosik KS. Primary cilia-autophagy-nrf2(pan) axis activation commits human embryonic stem cells to a neuroectoderm fate. Cell. 2016;165(2):410–420.
36. Zeng W, Xiao J, Zheng G, Xing F, Tiope GL, Wang X, He C, Chen ZY, Liu Y. Antioxidant treatment enhances human mesenchymal stem cell anti-stress ability and therapeutic efficacy in an acute liver failure model. Sci Rep. 2015;5:11100.
37. Drowley L, Okada M, Aebersold R, et al. Nad(+) repletion improves mitochondrial and stem cell function and enhances life span in mice. Science. 2016;352(6292):1436–1443.
38. Chen et al.
signaling in a mammalian model of accelerated aging. Science. 2007;317(5839):803–806.

41. Brack AS, Conboy MJ, Roy S, Lee M, Kuo CJ, Keller C, Rando TA. Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. Science. 2007;317(5839):807–810.

42. Meshorer E, Gruenbaum Y. Gone with the Wnt/Notch: stem cells in laminopathies, progeria, and aging. J Cell Biol. 2008;181(1):9–13.

43. Zhang DY, Wang HJ, Tan YZ. Wnt/beta-catenin signaling induces the aging of mesenchymal stem cells through the DNA damage response and the p53/p21 pathway. PLoS One. 2011;6(6):e21397.

44. Florian MC, Nattamai KJ, Dorr K, Marka G, Eckl C, Andrea I, Schiemann M, Oostendorp RA, et al. A canonical to non-canonical wnt signalling switch in hematopoietic stem-cell ageing. Nature. 2013;503(7476):392–396.

45. Chen JR, Lazarenko OP, Shankar K, Blackburn ML, Badger TM, Ronis MJ. A role for ethanol-induced oxidative stress in controlling lineage commitment of mesenchymal stromal cells through inhibition of Wnt/beta-catenin signaling. J Bone Miner Res. 2010;25(5):1117–1127.

46. Han ES, Muller FL, Perez VI, Qi W, Liang H, Xi L, Fu C, Doyle E, Hickey M, Cornell J, et al. The in vivo gene expression signature of oxidative stress. Physiol Genomics. 2008;34(1):112–126.

47. Brady CA, Attardi LD. P53 at a glance. J Cell Sci. 2010;123(Pt 5):e201.

48. Gambino V, De Michele G, Venezia O, Migliaccio P, Dall’Olio V, Flori JM, Vina J, Blasco MA, Serrano M. Delayed ageing and exhibit epigenetic dysregulation. PLoS Biol. 2007;5(8):e213.

49. Gharibi B, Farzadi S, Ghuman M, Hughes FJ. Inhibition of Akt/mTOR attenuates age-related changes in mesenchymal stem cells. Stem Cells. 2014;32(8):2256–2266.

50. Taniguchi Ishikawa E, Gonzalez-Nieto D, Ghiaur G, Dunn SK, Ficker AM, Murali B, Madhu M, Gutstein DE, Fishman GI, Barrio LC, et al. Connexin-43 prevents hematopoietic stem cell senescence through transfer of reactive oxygen species to bone marrow stromal cells. Proc Natl Acad Sci USA. 2012;109(23):9071–9076.

51. Arslan F, Lai RC, Smeets MB, Akeroyd L, Choo A, Agour EN, Timmers L, van Rijen HV, Doevendans PA, Pasterkamp G, et al. Mesenchymal stem cell-derived exosomes increase apoptosis levels, decrease oxidative stress and activate p38/mTor pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. Stem Cell Res. 2013;10(3):301–312.

52. Xu J, Qian J, Xie X, Lin L, Zou Y, Fu M, Huang Z, Zhang G, Su Y, Ge J. High density lipoprotein protects mesenchymal stem cells from oxidative stress-induced apoptosis via activation of the p38/mTor pathway and suppression of reactive oxygen species. Int J Mol Sci. 2012;13(12):17104–17120.

53. Bhattacharyya S, Dudeja PK, Tobacman JK. ROS, Hsp27, and Akt/mTOR in the response to genotoxic stress and regulating lifespan. Int J Biochem Cell Biol. 2008;40(2):176–180.

54. Liu Q, Li Y, Jiang W, Li Y, Zhou L, Song B, Liu X. Inhibition of HSP90 promotes neural stem cell survival from oxidative stress through attenuating NF-kappaB/p65 activation. Oxid Med Cell Longev. 2016;2016:3507290.

55. Shintaku J, Peterson JM, Talbert EE, Gu JM, Ladner KJ, Williams DR, Mousavi K, Wang R, Sartorelli V, Guttridge DC. Myod regulates skeletal muscle oxidative metabolism through inhibition of Wnt/beta-catenin signaling. J Bone Miner Res. 2010;25(5):1117–1127.
cooperatively with alternative nF-kappab. Cell Rep. 2016; 17(2):514–526.
69. Deng P, Zhou C, Alvarez R, Hong C, Wang CY. Inhibition of ikk/nF-kappab signaling enhances differentiation of mesenchymal stromal cells from human embryonic stem cells. Stem Cell Rep. 2016;6(4):456–465.
70. Arthur JS, Ley SC. Mitogen-activated protein kinases in innate immunity. Nat Rev Immunol. 2013;13(9):679–692.
71. Bose C, Bhuvaneswaran C, Udupa KB. Age-related alteration in hepatic acyl-coa: cholesterol acyltransferase and its relation to ldl receptor and mapk. Mech Ageing Dev. 2005;126(6–7):740–751.
72. Williamson D, Gallagher P, Harber M, Hollon C, Trappe S. Mitogen-activated protein kinase (mapk) pathway activation: effects of age and acute exercise on human skeletal muscle. J Physiol. 2003;547(Pt 3):977–987.
73. Rice KM, Desai DH, Preston DL, Wehner PS, Blough ER. Uniaxial stretch-induced regulation of mitogen-activated protein kinase, Akt and p70 S6 kinase in ageing fischer 344 x Brown Norway rat aorta. Exp Physiol. 2007;92(5):963–970.
74. Zeng S, Feirt N, Goldstein M, Guarrera J, Ippagunta N, Ekong U, Tian L, Hu Y, Schmidt AM, et al. Blockade of receptor for advanced glycation end product (rage) attenuates ischemia and reperfusion injury to the liver in mice. Hepatology. 2004;39(2):422–432.
75. Ito K, Hirao A, Arai F, Takubo K, Matsuoka S, Miyamoto K, Ohmura M, Naka K, Hosokawa K, Ikeda Y, et al. Reactive oxygen species act through p38 mapk to limit the lifespan of hematopoietic stem cells. Nat Med. 2006;12(4):446–451.
76. Bernet JD, Doles JD, Hall JK, Kelly Tanaka K, Carter TA, Olwin BB. P38 mapk signaling underlies a cell-autonomous loss of stem cell self-renewal in skeletal muscle of aged mice. Nat Med. 2014;20(3):265–271.
77. Sun Z, Huang Z, Zhang DD. Phosphorylation of Nrf2 at multiple sites by map kinases has a limited contribution in modulating the Nrf2-dependent antioxidant response. PLoS One. 2009;4(8):e6588.
78. Gutierrez-Uzquiza A, Arechederra M, Bragado P, Aguirre-Ghiso JA, Porras A. P38alpha mediates cell survival in response to oxidative stress via induction of antioxidant genes: effect on the p70s6 k pathway. J Biol Chem. 2012;287(4):2632–2642.
79. Chen H, Liu X, Chen H, Cao J, Zhang L, Hu X, Wang J. Role of SIRT1 and AMPK in mesenchymal stem cells differentiation. Ageing Res Rev. 2014;13:55–64.
80. Yuan HF, Zhai C, Yan XL, Zhao DD, Wang JX, Zeng Q, Chen L, Nan X, He LJ, Li ST, et al. SIRT1 is required for long-term growth of human mesenchymal stem cells. J Mol Med. 2012;90(4):389–400.
81. Zhang F, Cui J, Liu X, Lv B, Liu X, Xie Z, Yu B. Roles of microrna-34a targeting sirt1 in mesenchymal stem cells. Stem Cell Res Ther. 2015;6:195.
82. Liu J, Han W, Chen L, Tang K. Mechanism of osteogenic and adipogenic differentiation of tendon stem cells induced by sirtuin 1. Mol Med Rep. 2016;14(2):1643–1648.
83. Feng G, Zheng K, Song D, Xu K, Huang D, Zhang Y, Cao P, Shen S, Zhang J, Feng X, et al. SIRT1 was involved in tnf-
alpha-promoted osteogenic differentiation of human dpscs through wnt/beta-catenin signal. In Vitro Cell Dev Biol Anim. 2016;52(10):1001–1011.
84. Si X, Chen W, Guo X, Chen L, Wang G, Xu Y, Kang J. Activation of GSK3beta by Sirt2 is required for early lineage commitment of mouse embryonic stem cell. PLoS One. 2013;8(10):e76699.
85. Brown K, Xie S, Qiu X, Mohrin M, Shin J, Liu Y, Zhang D, Scadden DT, Chen D. Sirt3 reverses aging-associated degeneration. Cell Rep. 2013;3(3):319–327.
86. Wang QX, Shao Y, Ma CY, Chen W, Sun L, Liu W, Zhang DY, Fu BC, Liu KY, Jia ZB, et al. Decreased sirt3 in aged human mesenchymal stromal/stem cells increases cellular susceptibility to oxidative stress. J Cell Mol Med. 2014;18(11):2298–2310.
87. Son MJ, Kwon Y, Son T, Cho YS. Restoration of mitochondrial nad+ levels delays stem cell senescence and facilitates reprogramming of aged somatic cells. Stem Cells. 2016;34(12):2840–2851.
88. Mostoslavsky R, Chua KF, Lombard DB, Pang WW, Fischer MR, Gellon L, Liu P, Mostoslavsky G, Franco S, Murphy MM, et al. Genomic instability and aging-like phenotype in the absence of mammalian Sirt6. Cell. 2006;124(2):315–329.
89. Kanfi Y, Naiman S, Amir G, Peshti V, Zinman G, Nahum L, Bar-Joseph Z, Cohen HY. The sirtuin sirt6 regulates lifespan in male mice. Nature. 2012;483(7388):218–221.
90. Pan H, Guan D, Liu X, Li J, Wang L, Wu J, Zhou J, Zhang W, Ren R, Zhang W, et al. Sirt6 safeguards human mesenchymal stem cells from oxidative stress by coactivating nrf2. Cell Res. 2016;26(2):190–205.
91. Wang H, Diao D, Shi Z, Zhu X, Gao Y, Gao S, Liu X, Wu Y, Rudolph KL, Liu G, et al. Sirt6 controls hematopoietic stem cell homeostasis through epigenetic regulation of wnt signaling. Cell Stem Cell. 2016;18(4):495–507.
92. Sun H, Wu Y, Fu D, Liu Y, Huang C. Sirt6 regulates osteogenic differentiation of rat bone marrow mesenchymal stem cells partially via suppressing the nuclear factor-kappab signaling pathway. Stem Cells. 2014;32(7):1943–1955.
93. Tang YL, Zhou Y, Wang YP, Wang JW, Ding JC. Sirt6/nf-kappab signaling axis in ginsenoside rgl-delayed hematopoietic stem/progenitor cell senescence. Int J Clin Exp Pathol. 2015;8(5):5591–5596.
94. Liu J, Liu M, Ye X, Liu K, Huang J, Wang L, Ji G, Liu N, Tang X, Baltz JM, et al. Delay in oocyte aging in mice by the antioxidant n-acetyl-l-cysteine (nac). Hum Reprod. 2012;27(5):1411–1420.
95. Liu B, Ghosh S, Yang X, Zheng H, Liu X, Wang Z, Jin G, Zheng B, Kennedy BK, Suh Y, et al. Resveratrol rescues sirt1-dependent adult stem cell decline and alleviates progeroid features in laminopathy-based progeria. Cell Metab. 2012;16(6):738–750.
96. Tseng PC, Hou SM, Chen RJ, Peng HW, Hsieh CF, Kuo ML, Yen ML. Resveratrol promotes osteogenesis of human mesenchymal stem cells by upregulating runx2 gene
expression via the sirt1/foxo3a axis. J Bone Miner Res. 2011; 26(10):2552–2563.

97. Takacs-Vellai K, Vellai T, Puoti A, Passamante M, Wicky C, Streit A, Kovacs AL, Muller F. Inactivation of the autophagy gene bec-1 triggers apoptotic cell death in c. Elegans. Curr Biol. 2005;15(16):1513–1517.

98. Juhasz G, Erdi B, Sass M, Neufeld TP. Atg7-dependent autophagy promotes neuronal health, stress tolerance, and longevity but is dispensable for metamorphosis in drosophila. Genes Dev. 2007;21(23):3061–3066.

99. Watt MR, Binnewies M, Flach J, Reynaud D, Garg T, Malhotra R, Debnath J, Passegue E. Foxo3a directs a protective autophagy program in haematopoietic stem cells. Nature. 2013;494(7437):323–327.

100. Yilmaz OH, Katajisto P, Lamming DW, Gultekin Y, Bauer-Wuwke KE, Sengupta S, Birsoy K, Dursun A, Yilmaz VO, Selig M, et al. Mtorc1 in the paneth cell niche couples intestinal stem-cell function to calorie intake. Nature. 2012; 486(7404):490–495.

101. Chang KM, Yu SW. Interplay between autophagy and pro-apoptotic components regulates autophagy. PLoS One. 2015;10(5):e0126537.

102. Garcia-Prat L, Martinez-Vicente M, Perdiguero E, Ortet L, Gurny S, et al. Autophagy promotes survival, differentiation and function of neural stem cells. EMBO J. 2014;33(23):2782–2797.

103. Hammond SM, Sharpless NE. Hmga2, micrornas, and stem cell aging. Cell. 2008;135(6):1013–1016.

104. Guo YL, Chakraborty S, Rajan SS, Wang R, Huang F. Effects of oxidative stress on mouse embryonic stem cell proliferation, apoptosis, senescence, and self-renewal. Stem Cells Dev. 2010;19(9):1321–1331.

105. Takacs-Vellai K, Vellai T, Puoti A, Passamante M, Wicky C, Streit A, Kovacs AL, Muller F. Inactivation of the autophagy gene bec-1 triggers apoptotic cell death in c. Elegans. Curr Biol. 2005;15(16):1513–1517.

106. Li CJ, Sun LY, Pang CY. Synergistic protection of autophagy promotes neuronal health, stress tolerance, and longevity but is dispensable for metamorphosis in drosophila. Genes Dev. 2007;21(23):3061–3066.

107. Garcia-Prat L, Martinez-Vicente M, Perdiguero E, Ortet L, Gurny S, et al. Mtorc1 in the paneth cell niche couples intestinal stem-cell function to calorie intake. Nature. 2012; 486(7404):490–495.

108. Hammond SM, Sharpless NE. Hmga2, micrornas, and stem cell aging. Cell. 2008;135(6):1013–1016.

109. Guo YL, Chakraborty S, Rajan SS, Wang R, Huang F. Effects of oxidative stress on mouse embryonic stem cell proliferation, apoptosis, senescence, and self-renewal. Stem Cells Dev. 2010;19(9):1321–1331.

110. Sharpless NE, DePinho RA. How stem cells age and why this makes us grow old. Nat Rev Mol Cell Biol. 2007;8(9): 703–713.

111. Yahata T, Takanashi T, Muguruma Y, Ibrahim AA, Matsu- zawa H, Uno T, Sheng Y, Onizuka M, Ito M, Kato S, et al. Accumulation of oxidative DNA damage restricts the self-renewal capacity of human hematopoietic stem cells. Blood. 2011;118(11):2941–2950.

112. Suzuki K, Murtuza B, Beauchamp JR, Smolenski RT, Varela-Carver A, Fukushima S, Coppen SR, Partridge TA, Yacoub MH. Dynamics and mediators of acute graft attrition after myoblast transplantation to the heart. FASEB J. 2004; 18(10):1153–1155.

113. He T, Peterson TE, Holmuhamedov EL, Terzie A, Caplice NM, Oberley LW, Katusic ZS. Human endothelial progenitor cells tolerate oxidative stress due to intrinsically high expression of manganese superoxide dismutase. Arterioscler Thromb Vasc Biol. 2004;24(11):2021–2027.

114. Choudhary J, Nigam A, Kanwar YS, Neufeld TP. Regulation of stem cell function in tissue homeostasis and organ- ismal ageing. Nat Cell Biol. 2016;18(8):823–832.

115. Li L, Guo Y, Zhai H, Yin Y, Zhang J, Chen H, Wang L, Li N, Liu R, Xia Y. Aging increases the susceptibility of mescs to reactive oxygen species and impairs their therapeutic potency for myocardial infarction. PLoS One. 2014;9(11): e111850.

116. Chao JT, Chiang HJ, Chen CH, Sung PH, Lee FY, Tsai TH, Chang CL, Chen HH, Sun CK, Leu S, et al. Melatonin treatment further improves adipose-derived mesenchymal stem cell therapy for acute interstitial cystitis in rat. J Pineal Res. 2014;57(3):248–261.

117. Cai J, Yu X, Zhang B, Zhang H, Fang Y, Liu S, Ding X. Promotion of survival and engraftment of transplanted adipose-derived stromal and vascular cells from adipose tissue-derived stromal and vascular cells by overexpression of manganese superoxide dismutase. Int J Mol Sci. 2016;17(7). pii: E1082. doi: 10.3390/ijms17071082.
124. Li X, Liu J, Lin J, Wang T, Huang J, Lin Y, Chen D. Protective effects of dihydromyricetin against *oh-induced mesenchymal stem cells damage and mechanistic chemistry. Molecules. 2016;21(5). pii: E604. doi: 10.3390/molecules21050604.

125. Kanninen KM, Pomesdchik Y, Leinonen H, Malm T, Koistinaho J, Levonen AL. Applications of the keap1-nrf2 system for gene and cell therapy. Free Radic Biol Med. 2015;88(Pt B):350–361.

126. Zhao C, Xiu Y, Ashton J, Xing L, Morita Y, Jordan CT, Boyce BF. Noncanonical nf-kappab signaling regulates hematopoietic stem cell self-renewal and microenvironment interactions. Stem Cells. 2012;30(4):709–718.

127. Kim SU, de Vellis J. Stem cell-based cell therapy in neurological diseases: a review. J Neurosci Res. 2009;87(10):2183–2200.

128. Chhabra P, Brayman KL. Stem cell therapy to cure type 1 diabetes: from hype to hope. Stem Cells Transl Med. 2013;2(5):328–336.

129. Artandi SE, Alson S, Tietze MK, Sharpless NE, Ye S, Greenberg RA, Castrillon DH, Horner JW, Weiler SR, Carrasco RD, et al. Constitutive telomerase expression promotes mammary carcinomas in aging mice. Proc Natl Acad Sci USA. 2002;99(12):8191–8196.

130. Perez VI, Bokov A, Van Remmen H, Mele J, Ran Q, Ikeno Y, Richardson A. Is the oxidative stress theory of aging dead? Biochim Biophys Acta. 2009;1790(10):1005–1014.

131. Dai DF, Chiao YA, Marcinek DJ, Szeto HH, Rabinovitch PS. Mitochondrial oxidative stress in aging and healthspan. Longev Healthspan. 2014;3:6.