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Redox Regulation of Stem/Progenitor Cells and Bone Marrow Niche

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Abstract

Bone marrow (BM)-derived stem and progenitor cell functions including self-renewal, differentiation, survival, migration, proliferation and mobilization are regulated by unique cell-intrinsic signals and -extrinsic signals provided by their microenvironment, also termed the 'niche'. Reactive oxygen species (ROS), especially hydrogen peroxide (H_2O_2), play important roles in regulating stem and progenitor cell function in various physiologic and pathologic responses. The low level of H_2O_2 in quiescent hematopoietic stem cells (HSCs) contributes to maintain their stemness, whereas a higher level of H_2O_2 within HSCs or their niche promotes differentiation, proliferation, migration, and survival of HSCs or stem/progenitor cells. Major sources of ROS are NADPH oxidase and mitochondria. In response to ischemic injury, ROS derived from NADPH oxidase are increased in the BM microenvironment, which is required for hypoxia and HIF1α expression and expansion throughout the BM. This, in turn, promotes progenitor cell expansion and mobilization from BM, leading to reparative neovascularization and tissue repair. In pathophysiological states such as aging, atherosclerosis, heart failure, hypertension and diabetes, excess amounts of ROS create an inflammatory and oxidative microenvironment, which induces cell damage and apoptosis of stem and progenitor cells. Understanding the molecular mechanisms of how ROS regulate the functions of stem and progenitor cells and their niche in physiological and pathological conditions will lead to the development of novel therapeutic strategies.

Introduction

Adult stem cells are populations of cells that are able to regenerate the multiple differentiated cell types of the organ in which they reside and self-renew themselves. Bone marrow (BM)-derived stem and progenitor cells play an important role in neovascularization, which is involved in normal development and wound repair, as well as various pathophysiologies such as ischemic heart disease and peripheral artery disease. This process depends on angiogenesis and vasculogenesis (*de novo* new vessel formation through BM-derived stem and progenitor cells) [1-4]. Hematopoietic stem cells (HSCs) are the most characterized adult stem cells which produce all types of immune cells and maintain blood production for their lifetime. A subpopulation of BM-derived cells called "endothelial progenitor cells" (EPCs) has been identified by their capacity to form endothelial-like cells *in vitro* and *in vivo* [1]. However, the definition of EPCs has recently been challenged, as this concept is still lacking of formal proof in the adult and even questioned in embryonic development [5]. Moreover, hematopoietic cells are shown to be derived from endothelial cells during embryonic development in the mouse [6]. Overall, BM-derived cells appear to have a bilineage potential and interconnection between hematopoietic and endothelial cells has been introduced as a new concept [7]. This is supported by various evidence that stem and progenitor cells in the BM including HSCs, EPCs and even myeloid progenitors contribute to neovascularization and tissue repair in various injury models. Moreover, BM-derived progenitor cells isolated with hematopoietic and endothelial makers have been tested in clinical trials, while further optimization is needed regarding their feasibility, safety and benefit in patients with cardiovascular diseases.

HSC and progenitor cell function and fate are regulated by cell-intrinsic signaling and extrinsic cues provided by a distinct microenvironment called the 'niche' [8-13]. In the steady state of homeostatic hematopoiesis or under stress conditions such as after irradiation, growth factor stimulation and hematopoietic injury by chemotherapeutic agents, the mechanism of these regulations has been investigated regarding self-renewal, survival, differentiation, proliferation, engraftment (homing from the periphery to the niche) and mobilization (the forced migration of

the cells out of the BM niche into the periphery). It is beginning to be understood about cell-intrinsic and cell-extrinsic effects on the functions of stem and progenitor cells which are involved in inflammation, neovascularization and tissue repair after injury or infection.

Reactive oxygen species (ROS) such as superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) play an important role for stem and progenitor functions. In general, there is a 'redox window' hypothesis; appropriate ROS production is required for physiological cellular functions, while excess ROS contribute to pathological conditions. There seems to be a clear correlation between intracellular H_2O_2 levels and functions in stem and progenitor cells [14-20]. A low level of endogenous H_2O_2 is involved in maintaining the quiescence of HSCs, whereas a higher level of H_2O_2 contributes to a greater proliferation, senescence or apoptosis, leading to a premature exhaustion of self-renewal in these cells [21,22]. Thus, keeping H_2O_2 at low level within the HSCs or stem cell niche is an important feature of stemness that is directly related to the relatively quiescent state of stem cells *in vivo*. H_2O_2 at physiological levels activate repair processes that involve recruitment and differentiation of stem/progenitor cells. ROS derived from NADPH oxidase are required for hypoxia and HIF1α expansion in the BM microenvironment in response to ischemic injury [23]. This, in turn, promotes progenitor cell expansion and mobilization from BM, leading to reparative neovascularization and tissue repair. In pathophysiological states such as aging, diabetes, hypertension, atherosclerosis and cardiac infarction, excess amounts of ROS are generated, thus creating an inflammatory and oxidant stress microenvironment, which induces cell damage and apoptosis of stem and progenitor cells.

In this review, we will summarize the recent progress regarding the role of ROS and ROS-mediated BM microenvironment in regulating stem and progenitor cell functions including self-renewal, differentiation, survival/apoptosis, proliferation, migration and mobilization. Given the significant role of BM-derived cells in physiological and pathological conditions, understanding the redox regulation of stem/progenitor cell function and BM niche will lead to the development of stem and progenitor cell- or stem cell niche-targeted therapies.

1. Role of ROS in physiological and pathological cellular function

In general, excess amounts of ROS are detrimental to cells and contribute to various pathologies such as atherosclerosis, heart failure, aging, diabetes and cancer. In contrast, ROS, especially H_2O_2, at physiological levels function as signaling molecules to mediate various biological responses such as cell proliferation, migration, survival, differentiation and gene expression [24-27]. Cellular ROS levels are temporally and spatially regulated by the fine-tuned balance between ROS generation system and antioxidant enzymes. ROS such as $O_2^{\bullet-}$ and H_2O_2 are generated from a number of sources including mitochondria, NADPH oxidases (NOXs), xanthine oxidase, cytochrome p450 and nitric oxide synthase (through its uncoupling). Since $O_2^{\bullet-}$ is produced from oxygen, oxygen concentration or hypoxic condition has a significant impact on total amount of ROS. The $O_2^{\bullet-}$ reacts with nitric oxide (NO) to generate peroxynitrite ($OONO^-$), thereby inhibiting endothelial function [28], while it can be quickly converted to H_2O_2 by superoxide dismutases (SODs) such as MnSOD, (SOD2) or Cu/ZnSODs (SOD1) or extracellular SOD (SOD3) [29,30]. H_2O_2 is catalyzed by catalase [31], glutathione peroxidases (GPx) [32] and Thioredoxin-peroxiredoxin (Trx-Prx) system [33] to non-reactive water (Figure 1). Since H_2O_2 is relatively stable and does not react with NO, it has been proposed to function as a second messenger in physiological redox signaling. Overall, the levels of ROS are determined by the balance of ROS generation and antioxidant enzyme activity. Harmful effects of ROS on the cells are DNA damage, lipid peroxidation, protein oxidation and inactivation of specific enzymes by oxidation of co-factors, linking to the pathological consequences. It is well known that growth factor signaling is mediated through H_2O_2 production. The biological effect of ROS in the cell is dependent on their amount and duration, their source and subcellular localization, and type of species (Figure 1). Identifying direct molecular target(s) of ROS in each cell type is important to understand the cellular mechanism of redox regulation.

NOX generates ROS by catalyzing electron transfer from NADPH to molecular oxygen, O_2. Phagocyte NADPH oxidase consists of 2 membrane-bound subunits, $gp91^{phox}$ (NOX2) and $p22^{phox}$ which form the flavocytochrome b_{558} complex, together with the cytosolic subunits

$p47^{phox}$, and $p67^{phox}$ $p40^{phox}$ and the small GTPase Rac. NOXs have several homologs of NOX2 including Nox1, Nox3, Nox4, and Nox5, as well as the Dual oxidases (Duox), Duox1 and Duox2 [34,35]. Different from phagocytic NADPH oxidase that is normally quiescent but generates a large burst of $O_2^{\cdot -}$ (the "oxidative burst") upon activation, the NOXs constitutively produce low levels of $O_2^{\cdot -}$ or H_2O_2 intracellularly in basal state and are further stimulated acutely by various agonists and growth factors. NOXs are now recognized to have specific subcellular localizations, which is required for localized H_2O_2 production and activation of specific redox signaling pathways to mediate various functions [27,36]. They are found in various cell systems including endothelial cells, hematopoietic cells, mesenchymal cells and stem cells, and regulate cell migration, proliferation, differentiation, apoptosis, senescence, inflammatory responses and oxygen sensing [34]. NOXs have been implicated in numerous physiologies such as angiogenesis, tissue repair, hematopoiesis and stem/progenitor functions [14,35,37] as well as pathophysiologies such as hypertension, atherosclerosis, cancer and immune disorders [34,38-41]. Of note, NOX2 is involved in not only host defense but also chemotaxis, immune responses, the initiation of antigen cross presentation[41], cell survival[42] as well as immunosuppressing function protecting from autoimmune development[40]. We have demonstrated that NOX2 in stem/progenitor cells including EPCs, and the BM niche play an important role in reparative neovascularization in response to ischemic injury [23,43]. This issue is discussed below.

ROS are also produced from mitochondria as a consequence of aerobic metabolism. Increasingly, mitochondrial oxidants are viewed less as byproducts of metabolism and act as signaling molecules [44]. Both complex I and III of the electron transport chain are thought to be the major sites of mitochondrial ROS production [45,46]. In addition, mutations in either mitochondrial DNA or nuclear DNA that lead to disruption in any of the components of the electron transport chain also promote ROS formation [44]. Ischemia and apoptosis are shown to trigger $O_2^{\bullet -}$ production by complex III [47]. Once $O_2^{\bullet -}$ is generated, it is immediately converted into H_2O_2 by MnSOD, (SOD2) or Cu/ZnSODs (SOD1) [29,30]. To avoid the potential damaging effects of H_2O_2, mitochondria express other antioxidant enzymes such as

peroxiredoxins (Prx)3 and Prx5 and glutathione peroxidase. A number of studies suggest that hypoxic conditions increase mitochondrial ROS production, which stabilizes HIF1α protein expression. Moreover, mitochondrial oxidants also act as important signaling molecules to regulate the inflammatory response, autophagy and mitophagy as well as stem cell function [44].

The cross-talk between NOXs and mitochondrial ROS has been reported in various cell systems [48,49]. Hypoxia-induced mitochondrial H_2O_2 activate NOX via protein kinase C epsilon in pulmonary artery smooth muscle cells [50]. Serum withdrawal-induced mitochondria H_2O_2 stimulate NOX1 through PI3-kinase-Rac1 axis in human 293T cells [51]. These evidences suggest that mitochondrial H_2O_2 regulate NOX activity. Conversely, NOX activation induces mitochondrial H_2O_2 formation by opening of mitochondrial ATP-sensitive potassium channels [48]. Given that H_2O_2 is highly diffusible molecule, cross-talk between NOXs and mitochondrial ROS may represent positive feed-forward mechanisms that promote sustained H_2O_2 production and activation of redox signaling. Whether this regulatory mechanism is involved in stem and progenitor cell function is the subject of future investigation. Moreover, not only intracellular H_2O_2 but also extracellular H_2O_2 also play an important role in signal transduction, because H_2O_2 is relatively stable and can across the cellular membrane through aquaporin [52], and exogenously added H_2O_2 can activate NOXs [50]. Thus, intracellular H_2O_2 levels and redox signaling can be affected by extracellular H_2O_2 and H_2O_2 production from surrounding cells (cell-extrinsic effect). Cell-intrinsic and cell-extrinsic effects of ROS on stem and progenitor cell function are discussed below.

2. Cell-intrinsic effects of ROS on stem and progenitor function

Physiologic induction of ROS in stem and progenitor cells is regulated by growth factor or cytokine stimulation, changes in oxygen and/or energy metabolism, cell status and differentiation. These ROS inducing factors and situations appear to closely link to one another (Figure 2). As illustrated in Figure 3, distinguishing cell-intrinsic and -extrinsic effect of H_2O_2 is not always possible due to their diffusible nature. However, studies have demonstrated a clear

correlation of ROS levels in stem and progenitor cells, as measured by redox-sensitive dyes which detect mainly intracellular H_2O_2 or $O_2^{\bullet-}$, with their functions or stage of differentiation (Table 1). When the differentiation capacity is examined in the HSCs based on their H_2O_2 levels, ROS^{high} cells show higher myeloid differentiation capacity, whereas ROS^{low} cells retain their long-term self-renewal ability [21]. Some of the gene mutations exhibit the abnormal increase in H_2O_2 level, which promotes HSCs to exit from quiescence, block the self-renewal capacity and promote stem cell differentiation [18,20]. Moreover, H_2O_2 level is further higher in myeloid committed progenitor cells compared to HSCs and mechanism of redox regulation within both cell types is different in terms of FoxO-dependency [20]. It has been shown that a low-oxygenic niche in bone marrow limits ROS production, thus providing long-term protection for HSCs from oxidative stress [10,53,54]. This suggests that there are cell-intrinsic (by programming) and passive (by cellular adaptation) regulation for ROS levels. In *Drosophila*, developmentally regulated, moderately high H_2O_2 levels in the hematopoietic progenitors promote differentiation through JNK and FoxO activation [55]. Thus, H_2O_2 has cell-intrinsic effects on HSCs and hematopoietic progenitor cells during normal hematopoiesis. Cell-intrinsic effects of H_2O_2 on stem and progenitor cell function under stress conditions have also demonstrated. ROS levels in EPCs (which could have hematopoietic potential) is lower than those in mature ECs, which is due to higher expression of antioxidant enzymes (MnSOD, catalase and glutathione peroxidase), and is required for preserving "stemness" such as undifferentiated, self-renewing state under oxidant stress [33].

Hematopoietic growth factors or cytokines stimulate signaling events leading to cell growth [56] or promote HSCs mobilization into the circulation [57] through the formation of H_2O_2. In addition, hyperbaric oxygen stimulates recruitment and differentiation of circulating stem/progenitor cells in subcutaneous Matrigel by increasing H_2O_2 [58]. Thus, H_2O_2 at appropriate levels contributes to proliferation and migration of HSCs (which links to mobilization from the BM to the circulation as well as homing to the target sites). By contrast, H_2O_2 at excess amount activates p38 MAPK to limit the lifespan and self-renewing capacity or

expansion of HSCs, resulting in premature senescence phenotype or apoptosis [18,59]. The concept of the redox window suggests that optimal levels of ROS are required for normal responses while excess or insufficient levels of ROS are associated with cellular dysfunction and reduced growth factor signaling, respectively [60]. This notion also seems to apply to the redox regulation of stem and progenitor cell function [22]. The relationship between the change in H_2O_2 levels in stem and progenitor cells and their functional consequence are summarized in Figure 4. Note that in most cases, the exact source of ROS in these cells is not clear, because of the difficulty of measuring ROS, which are diffusible and short-lived, in current available detecting methods.

3. Sources of ROS regulating stem and progenitor cell function

Although there are several sources of ROS, NOX and mitochondria are major ROS generating enzymes in BM-derived stem and progenitor cells. The connection between mitochondrial ROS and stem cell function has been indicated. Liu J et al. [61] demonstrated that mice lacking the Polycomb repressor Bmi-1 mice exhibit increase in mitochondrial dysfunction, reduced ATP, increased H_2O_2 levels, and subsequent DNA damage in BM cells. It has been shown that the tuberous sclerosis complex (TSC)-mammalian target of rapamycin (mTOR) pathway, a key regulator of cellular metabolism, maintains the quiescence and function of HSCs by repressing mitochondrial biogenesis and the production of H_2O_2 [62]. This result implicates the role of mitochondrial ROS in inhibiting stemness of HSCs. Low level of H_2O_2 production from mitochondria may be important in metabolic adaptation under conditions of low oxygen as well as regulating biological function of stem and progenitor cells. This point should be further clarified in future studies.

Because of availability of gene silencing and mutant mice for NOX enzymes, the role of NOX-derived ROS in stem and progenitor cell functions has been extensively investigated. In murine hematopoietic progenitor cells under homeostatic conditions, all NOXs are expressed [63]. Piccoli et al. showed that human G-CSF-mobilized $CD34^+$ HSCs express NOX1,

2 and 4, which generate low levels of ROS [64,65]. In their sequential study, the high resolution imaging of HSCs with the immunodetection of NOX indicates the presence of membrane 'rafts'-like microcompartments where the assembly/activation of the NOX components may be functionally integrated for creating redox signaling platforms [66]. They suggested that NOXs-derived ROS play an important role in differentiation from stem and progenitor cells. Among NOXs, NOX2 is most abundantly expressed in murine BM mononuclear cells [43] and, murine and human EPCs [67]. The important role of NOX2 is also demonstrated in the BM after ischemic tissue injury. NOX2 expression and ROS production are increased in BM mononuclear cells [43] and differentiated myeloid cells after hindlimb ischemia of mice [23], which stimulates stem progenitor cells expansion and mobilization from BM [23,43]. Nox2 is also involved in HGF- [68], hypoxia- [67] induced stem/progenitor cell mobilization from BM to peripheral blood. These studies indicate that NOX2-derived ROS mediate growth factor and chemokine signaling involved in progenitor cell and EPC migration, proliferation and survival under stress conditions. Thus, NOXs play a role in maintaining adequate ROS levels in HSC and hematopoietic/endothelial progenitor cells and contribute to their physiological function.

NOX-derived ROS are involved in maintenance of stemness and cardiovascular differentiation of embryonic stem (ES) cells. Undifferentiated self-renewing ES cells generate low level of endogenous ROS with low NOX enzyme expression, and NOX is dynamically regulated during ES cell differentiation to cardiomyocytes [69]. Endothelial differentiation from mouse ES cells involves ROS from NOX2, NOX1 and NOX4 [70,71], while smooth muscle cell differentiation is mediated by NOX4-derived H_2O_2 [72]. ROS generation is elevated during the early stages of ES cell differentiation, and then downregulated during later stages. During the differentiation process, anti-oxidative genes are downregulated [73] while NOX1, 2 and 4 are upregulated [74], thereby increasing ROS levels. Stimulation of ES cells with mechanical strain [71] or direct current electrical field [70,75,76] or low concentration of H_2O_2 [69,74] or various agonists including cardiotrophin-1 [77], PDGF-BB [78] or peroxisome proliferator-activated receptor α [79] induces cardiovascular differentiation of ES cells through increase of

H_2O_2. This in turn induces upregulation of NOX1 and 4, thus initiating a feed-forward stimulation of prolonged ROS generation [71,74]. Of note, NOX4 is involved in differentiation of ES cells to cardiomyocytes [80] and smooth muscle cells [72] while NOX2 is closely correlated to the differentiation of phagocytic cells from ES cells [81]. H_2O_2 derived from NOX activates ERK1/2, p38 and JNK [71], or various cardiogenic transcription factors [74], which are required for cardiomyogenesis of ES cells.

4. Regulators of ROS involved in stem and progenitor cell function

There are various regulators of ROS and their molecular targets involved in stem and progenitor cell function as described below (Figure 5).

Forkhead homeobox type O (FoxO):

The forkhead homeobox type O (FOXO) transcription factors FOXO1, FOXO3a, and FOXO4 are critical mediators of the cellular responses to oxidative stress and play a pivotal role in the redox regulation of HSCs [82]. FoxO3a$^{-/-}$ HSCs show increased phosphorylation of p38MAPK and H_2O_2 as well as downregulation of antioxidant enzymes, defective maintenance of quiescence, and heightened sensitivity to cell-cycle-specific myelotoxic injury [83]. It is shown that H_2O_2 act through p38MAPK to limit HSC lifespan [18]. Thus, excess H_2O_2-p38MAPK pathway may be involved in inhibition of self-renewal function of FoxO3a$^{-/-}$ HSCs. Furthermore, under stress conditions, such as aging or 5-FU-induced myelosuppression, FoxO3a$^{-/-}$ mice develop neutrophilia associated with increased Akt and ERK and decrease of Spred2 (Sprouty-related Ena/VASP homology 1 domain-containing proteins 2). Thus, FoxO3a plays a pivotal role in maintenance, integrity, and stress resistance of HSCs through negative feedback pathways for proliferation [84]. Conditional FoxO1/3/4 knockout mice exhibit myeloid lineage expansion and a marked decrease in lineage-negative Sca1^{+}, cKit^{+} (LSK) compartment and long-term repopulating activity that correlated with increased cell cycling, apoptosis and H_2O_2 in HSCs [20]. These FoxO-deficient HSC phenotypes are rescued by *in vivo* treatment with the

antioxidant N-acetyl-L-cysteine (NAC). Thus, FoxOs proteins protect against oxidative stress and thereby maintain self-renewal capacity (quiescence) and enhanced survival in the HSC compartment, which is required for its long-term regenerative potential [20].

Akt, PTEN and mTOR:

Akt phosphorylates FoxO to promote transition from quiescent status to myeloid-biased activated HSCs. HSCs isolated from $Akt1^{-/-}/Akt2^{-/-}$ mice show defective differentiation of HSCs into multipotent progenitors by decreasing H_2O_2 levels [85]. Constitutively active Akt accelerates proliferation and increases H_2O_2 level in HSCs, resulting in depletion of HSCs, BM failure as well as myeloproliferative disease or acute myeloid leukemia [86]. Thus, H_2O_2 is determinant factor for myeloid commitment and its appropriate level is important for normal HSC function. In addition, H_2O_2-mediated enhancements in self-renewal and neurogenesis are dependent on PI3K/Akt signaling [87]. PTEN is negative regulator for PI3K/Akt pathway and contains catalytic cysteine residues that are highly susceptible to oxidation by H_2O_2 [88,89]. Therefore, PTEN inhibition stabilizes the active phosphorylated form of Akt. Conditional $PTEN^{-/-}$ mice show rapid depletion of long-term repopulating HSCs and promoting myeloproliferative diseases, which are restored by mTOR inhibition [90]. Importantly, the depletion of PTEN-deficient HSCs is not mediated through ROS [91], while the defect by FOXO deficiency is rescued by NAC [20]. G-CSF-induced mobilization of hematopoietic progenitor cells into the circulation is mediated via increase in c-Met expression and its downstream mTOR-FOXO3a-mediated accumulation of H_2O_2 [57]. Therefore, H_2O_2-dependent and -independent mechanisms may be involved in the regulation of myeloid commitment in HSCs. Tuberous sclerosis complex (TSC) has been shown to regulate ROS levels in HSCs. *Tsc1* deletion in the HSCs drives them from quiescence into rapid cycling, with increased mitochondrial biogenesis and H_2O_2, and TSC-mediated decrease in H_2O_2 is mediated through mTOR inhibition[62]. Interestingly, the deletion of AMP kinase (*AMPK*), a downstream of TSC and a metabolic kinase, does not affect H_2O_2 levels [91]. Therefore, linkage between ROS and metabolic pathways appear to be complex, while both clearly regulate HSC functions.

ATM, p38MAPK and p53:

The 'ataxia telangiectasia mutated' (ATM) gene maintains genomic stability by activating a key cell-cycle checkpoint in response to DNA damage, telomeric instability or oxidative stress. Physiological levels of H_2O_2 are required to maintain genomic stabilities by activating the DNA repair pathway via ATM in cardiac and ES cells [92]. HSCs from $ATM^{-/-}$ mice have increased levels of H_2O_2 [17], leading to defective reconstitutive capacity of HSCs. Thus, the reduction of intracellular ROS levels by ATM is required for maintaining the self-renewal ability of HSCs. Mechanistically, H_2O_2 elevation due to ATM deficiency in HSCs activates p38MAPK, which upregulates the CDK inhibitors p16Ink4a and p19Arf [17]. Treatment with NAC rescues the defects in HSC function in ATM-deficient mice [17], suggesting that elevation of H_2O_2 can exit HSCs from quiescence and reduces self-renewal capacity [17,20,93]. Furthermore, overexpression of polycomb RING-finger oncogene BMI1 in normal human neural stem cells directly enhances ATM recruitment to sites of DNA damage, leading to protection from ultraviolet radiation [94] presumably by preventing generation of H_2O_2 [61]. Prdm16, a zinc finger transcription factor, is shown to be involved in the maintenance of stem cell function by modulating the intracellular redox state. Prdm16 deficiency increases mitochondrial H_2O_2 through BMI1, resulting in the depletion of stem cells, cell death and altered cell-cycle distribution [95]. In addition, ATM-mediated phosphorylation of BID, a BH3-only BCL2 family member, plays an important role in maintaining the quiescence and survival of HSCs via reducing oxidative stress [96]. Thus, the ATM-BID pathway serves as a critical checkpoint for coupling HSC homeostasis and the DNA-damage stress response to enable long-term regenerative capacity. Importantly, physiological levels of intracellular H_2O_2 are required to activate the DNA repair pathway for maintaining stem cell genomic stability [92]. This finding suggests that the concept of “redox window” or “oxidative optimum” can be also applied for genomic stability in stem cells.

p53, a major tumor suppressor gene, has been implicated in regulation of HSC quiescence and self-renewal. Activation of p53 depletes stem cells via H_2O_2 accumulation, and

Mdm2, an E3 ubiquitin ligase that targets p53 for degradation, is required for survival of HSCs/progenitors via dampening of H_2O_2-induced p53 activity [97]. Thus, excess activation of p53 in the absence of Mdm2 induces a dysregulated p53- H_2O_2 positive feedback loop, indicating that an appropriate level of p53 and H_2O_2 is essential for the maintenance of HSCs [98].

5. Role of BM niche in regulating stem and progenitor functions

Stem cells are localized to niches formed by cells that provide a microenvironment that provides essential cues supporting their growth and fate decisions in the BM [8,10,13,54,99,100]. Interaction of stem cells with the niche is crucial for the long-term maintenance of quiescence. HSC niche consists of sinusoidal endothelial cells (ECs), sympathetic nerve fibers, cells of the osteoblastic lineage, osteoclasts [101], perivascular mesenchymal stem cells [102], macrophages [103,104], regulatory T cells [105] and other HSC progenies [9] (Figure 6). In addition to these cellular components, soluble factors such as cytokines and growth factors, extracellular matrix, oxygen concentration or hypoxia, and ROS are also the part of the niche. Extrinsic instructions provided by unique microenvironments regulate the fate and functions of HSCs and progenitors. The number of studies on the niche are reviewed by others focusing on the mechanism of HSC maintenance (quiescence and self-renewal) [10], leukemia development and chemotherapy resistance [106,107], organization of niches [11,13], HSCs mobilization (HSC egress from the niche and trafficking into the blood) and homing to the BM [108-111].

6. Role of ROS, hypoxia and HIF1α in regulating BM niche

The stem cell niche includes the low oxygen endosteal niche mainly containing quiescent hematopoietic stem cells as well as more oxygenated vascular niche containing proliferative and differentiated hematopoietic progenitors [53,99,100]. This concept is coupled with the hypothesis of passive adaptation to regulate H_2O_2 generation in HSCs; a low-oxygen niche in BM limits H_2O_2 production, thereby providing long-term protection for HSCs from oxidant

stress. Thus, hypoxic microenvironment is an important determinant for the maintenance of ‘‘stemness”, and regulates stem cell self-renewal and differentiation [112]. *In vitro*, characteristics of undifferentiated HSCs such as colony-forming ability or reconstitution capacity are retained after hypoxic culture compared with normoxic conditions [113-115]. *In vivo*, Hoechst33342 dye perfusion assay reveals that long term-HSCs and a part of osteoblasts are found predominantly in poorly-perfused hypoxic niches in the BM compared to ECs and mesenchymal stem cells which show medium to high perfusion [116]. This suggests that the most quiescent HSCs localize in a region far from blood supply that delivers oxygen. The low ROS population has a higher self-renewal potential and reconstitution capacity following serial BM transplantation [21]. Thus, hypoxia and low blood perfusion seem to be correlated with low ROS level in HSCs in the BM. However, putative HSCs can also be localized at perivascular area of BM with low oxygen and high HIF1α expression [117]. Although it might be difficult to anatomically identify and define the hypoxic and relatively oxygenated microenvironment in the complex structure of BM [8], there seems to be at least theoretical hypoxic niche in the BM that leads to lower H_2O_2 production. Alternatively, the existence of hypoxic HSCs with perivascular localization may suggest that intracellular hypoxia can be achieved actively, not simply passively, by cell-intrinsic regulation through HIF1α [117].

In addition to the cell-intrinsic effects of H_2O_2 in HSCs, H_2O_2 produced from cells in the niche around the HSCs may act as extrinsic factors to regulate HSC functions. Cell-extrinsic effects of H_2O_2 are defined as HSC regulation through redox modification of niche components including cellular components, extracellular matrix and soluble factors (Figure 3). Of note, the diffusible nature of H_2O_2 and the difficulty of its measurement *in situ* often prevent investigators from identifying an action point of H_2O_2. Several evidence suggests that the osteoblasts, cells of mesenchymal origin positioned at the endosteal surface of bone, are essential components of the HSC niche [99]. Tie2/Angiopoietin1 (Ang1) signaling is required for the maintenance of HSCs in a quiescent state in the BM niche [17]. The role of N-cadhernin in the BM niche and regulation of HSC is a point of controversy [118-120]. Regardless, increase of H_2O_2 in HSCs by

anti-cancer drug has been reported to suppresse N-cadherin expression in osteoblastic niche and to induce shift of side population (SP) cells to non-SP cells, allowing quiescent HSCs detached from the niche [83]. Redox-dependent expression of vascular cell adhesion protein 1 on ECs in the BM is required for the early stages of BM homing and localization of HSC after irradiation [121]. Autocrine factors from Akt-mTOR-activated ECs support the self-renewal of long-term (LT)-HSCs (which contribute to hematopoiesis for long term) and expansion of hematopoietic stem and progenitor cells (HSPC), whereas MAPK co-activation favors maintenance and lineage-specific differentiation of HSPCs [122]. EC-derived growth factors support *in vitro* self-renewal and *in vivo* repopulation of authentic LT-HSCs through Notch [122]. Although further mechanistic studies are required, these reports suggest that ROS-mediated osteoblast or EC modification may regulate HSCs-niche interaction, resulting in modulating HSC function (Figure 3). Most recently, Taniguchi et al. have shown that hematopoietic connexin-43 prevents HSC senescence by reducing ROS level in HSCs through transferring ROS to BM stromal cells [123]. This finding indicates the novel mechanism for the niche-mediated regulation of ROS levels in HSCs.

BM macrophages maintain the endosteal niche and their depletion by G-CSF and clodronate-loaded liposomes induce HSC mobilization into the blood [103]. Similarly, specific depletion of $CD169^{+}$ macrophages localized in mesenchymal niche by a CXCR4 antagonist or G-CSF induces egress of HSC/progenitor cells [104]. Extensive studies are required to understand the niche regulation under stress conditions such as inflammation, in which HSC is differentiated into immune cells, and HSC and progenitor cells are mobilized from the BM to the blood circulation [124]. Thus, modulating the BM stem cell niche is important for developing novel therapeutic strategies [125,126].

Most recently, we performed *in vivo* injection of $O_2^{\bullet-}$ reactive probe and hypoxic bioprobe into mice and showed that ischemic injury increases ROS through NOX2 predominantly at the central BM *in situ* and at lesser extent at the endosteal regions [23]. Of note, NOX2-derived ROS are increased mainly in differentiated myeloid cells in the BM, thereby

creating an oxidative BM microenvironment. We also showed that ischemic injury induces expansion of low oxygen (hypoxic) areas throughout the BM, in a NOX2-dependent manner. This in turn regulates HSPCs expansion, survival and mobilization from the BM, leading to neovascularization and tissue repair [23] (Figure 7). Consistently, granulocyte colony stimulating factor (G-CSF) or cyclophosphamide (CY), which stimulates mobilization of stem/progenitor cells, increase hypoxia in the BM [127]. Of note, G-CSF and CY-induced stem cell mobilization is blunted in $NOX2^{-/-}$ mice [128]. Fan et al. showed that increase in ROS is associated with a decreased oxygen percentage in $CD34^{+}$ HSPCs [129]. Piccoli et al. [64] reported that the half of the oxygen consumption in HSPCs is dependent on NOX. In addition, the consumption of respiratory burst by NOX2 in differentiated myeloid cells is shown to increase local hypoxia [130]. Thus, it is likely that ischemic injury increases oxidative microenvironment mainly due to activation of NOX2 in differentiated myeloid cells, which in turn creates hypoxic niche throughout the BM by increasing oxygen consumption. These ROS-hypoxia-mediated alterations of the BM niche induced by inflammation or tissue injury may regulate stem and progenitor expansion and mobilization from BM, thereby promoting tissue repair and regeneration (Figure 3) [23]. Of note, hypoxia culture (2% O_2) rather suppresses HSC proliferation under growth factor stimulation regardless of NOX2 expression (N.U. and MU-F. unpublished observation). This suggests that above mentioned NOX2-ROS-mediated increase in hypoxic microenvironment is achieved in more oxygenic condition to promote progenitor cell expansion.

HIF1α is a key regulator of hypoxia, metabolic and angiogenic response. HIF1α is highly expressed in LT-HSCs [117,131]. HSCs derived from conditional HIF1α knockout mice exhibit impaired reconstitution capacity [117]. HSCs utilize glycolysis instead of mitochondrial oxidative phosphorylation to meet their energy demands through HIF1α [131]. This anaerobic-biased energy metabolism promotes HSC maintenance by limiting ROS production [54]. Activation of HIF1α or treatment with the HIF stabilizer reduces HSC reconstituting ability under normoxic conditions [15], which is supported by the study using the genetic mouse

model [117]. In *C. elegans*, mild reduction in mitochondrial respiration leads to the increase in H_2O_2 and HIF1α that are required for the acquisition of a long-life span [132]. These indicate that appropriate levels of HIF1α and H_2O_2 are responsible for the maintenance of HSCs and other stem cells. Moreover, NOX-derived ROS contribute to HIF1α stabilization in HSCs in normoxic conditions by down-regulation of the tumor suppressor von Hippel-Lindau protein (pVHL) [65]. Thus, HIF1α and ROS closely work together, along with oxygen homeostasis and energy metabolism, to maintain HSC function. In the stress response of stem and progenitor cells to ischemic injury, HIF1α in BM-derived cells promote angiogenesis [133]. *Ex vivo* cultured BM-derived angiogenic cells treated with the prolyl-4-hydroxylase inhibitor, which increases HIF1α and HIF2α expression, improves angiogenesis of ischemia hindlimb in old mice [134]. We have demonstrated that endosteum at the BM is hypoxic with high expression of HIF1α in basal state. In response to ischemia, NOX2-derived ROS are increased in both the endosteal and central region of BM tissue, which promotes HIF1α and VEGF expression with expansion of hypoxic areas in the BM *in situ* [23](Figure 7). Thus, NOX-ROS-mediated BM niche modification by ischemic injury may regulate hypoxia response in BM progenitor cells, promoting their mobilization from BM.

7. Other ROS-dependent regulators in the BM niche

ROS are involved in niche-mediated growth factor/chemokine receptor signaling through regulating its ligand expression. SDF-1α, which plays a role in stem and progenitor cell mobilization and vascular repair, regulates the trafficking of HSCs progenitors and maintaining HSC niches in BM [135]. SDF-1α is released by stromal cells and binds to its CXCR4 receptor on stem and progenitor cells. The high SDF-1α content in the BM creates a concentration gradient, which retains HSCs within the stem cell niche. Disruption of this SDF-1α gradient promotes mobilization of stem cells into the circulation, which occurs after upregulation of G-CSF levels during systemic stress or injury. In response to ischemia, myocardial infarction or hypoxia, tissue levels of SDF-1α are increased [136-139], which may attract stem cells to sites

of tissue injury and ischemia. The SDF-1-CXCR4 axis induces cMet activation in the BM, which promotes G-CSF-induced mobilization of progenitor cells via increasing ROS [57]. Most recently, Golan et al. reported that sphingosine-1 phosphate (S1P) promotes hematopoietic progenitors and BM stromal cell mobilization as well as SDF-1 release via ROS [140]. Thus, dynamic cross-talk between S1P and SDF-1 via ROS signaling integrates BM stromal cells and hematopoietic progenitor cell motility.

Ischemic injury increases cytokines and VEGF in the BM and circulation, which in turn activates matrix metalloproteinase (MMP)-9 and releases soluble Kit ligand in the BM [2]. MMPs including MMP-9, which is secreted mainly by neutrophils in BM [141], and MT (membrane type)1-MMP [142], which is anchored on the cell surface, plays a significant role in stem/progenitor cell mobilization and angiogenesis. We have demonstrated that NOX2-derived ROS increased in the BM after ischemic injury regulate HSPCs function in part through regulating Akt activation, expression of MT-1-MMP, and MMP-9 activity. Therefore, ROS regulate extracellular matrix in the BM niche. Taken together, understanding mechanisms by which ischemic injury regulates BM microenvironment is essential for developing novel therapeutic strategies for various ischemic diseases.

8. Role of ROS in stem and progenitor cell function in pathological conditions

In pathological conditions such as aging, atherosclerosis and diabetes, excess amount of ROS (oxidative stress) in stem and progenitor cells as well as BM microenvironment may impair stem and progenitor function, which can inhibit HSC self-renewal and induce HSC senescence, resulting in premature exhaustion of HSCs and hematopoietic dysfunction. Recent proteomic analysis of BM stromal cells in culture reveals that older stromal cells produce more H_2O_2 than younger cells [143]. Thus, both intrinsic dysregulation of ROS and more oxidative environment may have deleterious effects on stem and progenitor function. Although the definitions of EPCs has been challenged [7,16], inverse correlation between circulating number of EPCs and cardiovascular risk has been shown [46,144-154]. In human EPC, angiotensin II accelerates EPC

senescence through induction of oxidative stress [148]. Diabetes induces dysfunction and early senescence in stem and progenitor cells. In animal model of type I diabetes, $O_2^{\bullet-}$ production by eNOS uncoupling leads to reduction of EPC levels and impairment of EPC function [155]. In EPC culture, high glucose promotes EPC proliferation at early stage (3 days) and inhibits at later phase (7 days) through H_2O_2 accumulation [156]. The p66Shc deletion rescues the BM-derived EPCs defect induced by oxidative stress in high glucose [157]. Human EPCs from type II diabetes exhibit impaired proliferation, adhesion and incorporation into vascular structures [3]. Decreasing $O_2^{\bullet-}$ restores defective ischemia-induced new vessel formation induced by the glyoxalase 1 substrate methylglyoxal-mediated modification of HIF1α in EPCs [158], indicating a causal role of ROS in EPCs dysfunction in diabetes.

Circulating progenitors from healthy subjects have lower levels of H_2O_2 due to higher expression of the antioxidants enzymes including MnSOD, GPx, and catalase compared with human umbilical vein ECs [33]. Indeed, dysfunction of antioxidant defenses links to impaired function of EPCs; GPx-$1^{-/-}$ mice have no increase in circulating EPCs in response to either VEGF treatment or ischemic injury. GPx-$1^{-/-}$ EPCs are functionally deficient in promoting angiogenesis *in vivo* and *in vitro*, and show an increased susceptibility to oxidative stress *in vitro* [159]. Apoptosis signal-regulating kinase 1 (ASK1) is controlled by multiple redox-sensitive proteins including thioredoxin, glutathione-S-transferases, and glutaredoxin [16]. Ingram et al. showed that H_2O_2-induced increase in ASK1 activity is involved in diminished vessel-forming ability of EPCs after oxidant stress [160]. Moreover, decreased circulating progenitor cells and their dysfunction are associated with inflammation [154]. In addition, the H_2O_2-p38MAPK pathway accelerates senescence of EPCs by inducing pro-senescence molecule p16(INK4a) [161] in the same manner with quiescent HSCs. This indicates that HSC and EPC, or their progeny share the common pathway regarding premature senescence through excess amount of ROS.

9. Therapeutic potential of redox regulation of stem/progenitor cells and their niche

For a last decade, cytokines, chemokines and growth factors, which promote stem and progenitor egress from their niche or the mobilization from BM to the circulation, have been tested for their therapeutic potential in patients with cardiovascular diseases. The clinical trials of cell therapy using BM progenitor cells demonstrate its feasibility, safety and potential benefit in patients with ischemic disease and heart failure, but reveal that current cell-based therapy needs to be optimized to improve therapeutic efficacy [162]. In this regard, studies investigating the therapeutic potential of redox regulation of these cells have used two different strategies. The first approach is suppressing excess oxidative stress in stem and progenitor cells. Experimentally, EPC dysfunction prevents new blood vessel growth, which is restored by manipulations to decrease ROS. *In vivo* administration of SOD mimetic attenuates the diabetes-related impairment of BM mononuclear cells by reducing oxidative stress [163]. Thus, strategies aimed at reducing hyperglycemia-induced ROS is a useful antihyperglycemic therapies in the restoration of vasculogenesis and the prevention of diabetic complications [164]. Either transgenic expression of MnSOD or administration of SOD mimetic rescue impaired post-ischemic neovascularization and tissue survival [158]. Angiotensin II receptor and β1-adrenoceptor blockers improve the EPC dysfunction in hypertension via an antioxidant effect [165,166]. Treatment with organic nitrates increases circulating EPC levels, while increased NOX-derived ROS by isosorbide dinitrate induces their dysfunction [167,168]. Mesenchymal stem cell engraftment in the infarct heart is enhanced by anti-oxidant NAC co-injection [169]. Hypoxic preconditioning increases the survival and angiogenic potency of peripheral blood mononuclear cells through oxidative stress resistance mechanisms [170]. As a second approach, on the contrary, stimulating progenitor cells with controlled pro-oxidant has also shown to be effective on promoting their neovascular function. For example, short-term pretreatment with low-dose H_2O_2 enhances the efficacy of BM cells for therapeutic angiogenesis [171]. Injection of BM cells from control mice, but not NOX2-deficient mice, promotes neovascularization in response to tissue ischemia [43], suggesting that NOX2-derived ROS in BM cells is required for this response. *In vitro* preconditioning that stimulates mitochondrial H_2O_2 production increases the secretion of pro-

angiogenic properties from adipose-derived stroma cells and the survival of these cells in ischemic tissues after *in vivo* injection [172]. This suggests that mitochondrial H_2O_2 generation in stromal cells provides essential cues for stem and progenitor cells to promote neovascularization after injury.

There is doubled-edge effect of ROS whereby physiological levels can serve as signaling molecules to promote vascular integrity [43,173], whereas excess ROS levels in pathological conditions are associated with stem/progenitor dysfunction and/or impaired post-ischemic neovascularization [172,174-176]. Thus, antioxidant therapy in pathological conditions should be carefully designed so that ROS levels are kept optimal and physiological levels in BM stem/progenitor cells and microenvironment. Alternatively, more specific approaches by targeting particular ROS generating or antioxidant systems, or a downstream of ROS-sensitive molecules in stem and progenitor cells may be more effective as a new potential therapy.

Finally, modulating the stem and progenitor niche *in vivo* would have therapeutic potential for inflammatory- or ischemia-related cardiovascular diseases and this may allow us to stimulate stem and progenitor cells in the longer term. It has been shown that a defective niche results in HSC disorders, further emphasizing the important function of the HSC niche *in vivo* [177,178]. For a last decade, cytokines, chemokines and growth factors, which promote mobilization of stem and progenitor cells from the niche, have been tested for their therapeutic potential in patients with cardiovascular diseases, while their benefits seem to be relatively limited. As described above, mice lacking essential components of the regulatory system that maintains ROS within the physiological levels, show accelerated HSCs senescence and progressive BM failure [17,20]. In type 1 diabetic mice, the elevation in mitochondrial ROS induces stem/progenitor cell depletion and dysfunction in the BM microenvironment [179]. Thus, targeting against excess levels of ROS in the BM niche or the niche components may provide new therapeutic strategies for treatment of various cardiovascular diseases.

Conclusions

The current review outlines that ROS and ROS-mediated BM niche are involved in stem and progenitor cell functions including self-renewal, differentiation, survival/apoptosis, proliferation, and mobilization. ROS levels in stem and progenitor cells have a clear correlation with cellular functions and are regulated by a fine tuning of the balance between ROS generating and anti-oxidative defense systems. Molecular targets of ROS and distinct redox signaling pathways in stem and progenitor cells have been identified with *in vitro* and *in vivo* functional consequences. ROS are also considered as niche factors which regulate stem and progenitor cells through modulating other cellular and non-cellular niche components. The role of ROS in niche modification is beginning to be investigated. Because of the complexity of the BM niche, the diffusible nature of H_2O_2 and the difficulty of their tracking, it could be challenging to elucidate a dynamic regulation of the BM niche, especially in the pathophysiological conditions such as aging, metabolic disorders, inflammation, response to injury or infection, and autoimmune diseases. However, with the combination of advanced *in vitro*, *in vivo* and *ex vivo* techniques, we will be able to extract important elements for redox regulation of stem and progenitor cells, which may develop novel cell-based and/or niche-targeted therapies.

The niche engineering will be useful to test a hypothetical model and can be directly applied to cell therapy manufacturing that produces beneficial cell populations for regenerative medicine. This interplay may discriminate between pathways resulting in oxidative stress, and induction of apoptosis versus signaling events in stem and progenitor cells. ROS promote HSCs to exit from the self-renewal capacity and function as signaling molecules to promote stem cell differentiation into multi-lineage and larger homing capacity. This may contribute to angiogenic and/or tissue repair function of BM stem and progenitor cells. These mechanisms are regulated by the intrinsic redox control in stem and progenitor cells through various redox signaling pathways as well as by the extrinsic factors generated from the BM niche such as ROS, hypoxia, and cytokines/chemokines. NOX-ROS-mediated hypoxic BM microenvironment induced by ischemic injury increases HIF1α and VEGF expression in BM as well as progenitor cell survival and expansion, thereby promoting their mobilization from BM. Understanding the redox

regulation of stem and progenitor cells and BM niche as well as their underlying mechanisms in physiological and pathological conditions will lead to the development of novel therapeutic strategies.

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Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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Table

Table 1. Relationship between ROS levels and cellular functions in HSCs, and hematopoietic or endothelial progenitors

Description of cells	Isolation method (Source of Cell)	Change in ROS level (method used to measure)	Function(s)	Reference
HSPCs	Sca-1+/c-Kit+/Lin- (BM)	Increased by S1P (DHE)	motility, mobilization from the BM into the circulation	[140]

HSPCs	Sca-1+/c-Kit+/Lin- (BM)	Increased by G-CSF or HGF *in vivo* (DHE)	motility, mobilization from the BM into the circulation	[57]
LT-HSCs	CD34-/Flt3-/Sca-1+/c-Kit+/Lin- (BM)	Increased by Paf deficiency (DCFDA)	loss of quiescence	[180]
BM-derived EPCs	lectin+/ac-LDL+ (cultured BM-MNCs at day 7)	Increased in cells from STZ-mice (MitoSOX)	Impaired Matrigel tube formation, adhesion, and migration	[181]
		Decreased by AMPK activation in cells from STZ-mice (MitoSOX)	Improve diabetes-impaired Matrigel tube formation, adhesion, and migration	
EPCs	Early outgrowth EPCs	Increased by HGF (Amplex Red in supernatant)	mobilization from the BM into the circulation	[68]
HPCs	CD34+ (human cord blood)	Increased by Ras transduction (Diogenes, DEPMPO spin-trap, and AmplexRed)	survival, growth factor-independent proliferation	[182]
BM-derived EPCs	lectin+/ac-LDL+ (cultured rat BM-MNCs at day 7)	Increased by AGEs (DCFDA)	Apoptosis, reduced migration, adhesion and proliferation	[183]
HSPCs	Drosophila lymph gland	Increased by differentiation under *in vivo* physiological conditions	differentiation	[55]
BM-derived EPCs	lectin+/ac-LDL+ (cultured cKit+ BM-MNCs at day 7)	Increased by high glucose (DCFDA and DHE)	Apoptosis	[157]
BM-derived EPCs	lectin+/ac-LDL+ (cultured BM-MNCs at day 4)	Increased by hemin (DCFDA)	migration, proliferation and differentiation	[184]

BM-MNCs	Density gradient centrifugation (BM)	Increased in db/db mice (DCFDA)	Reduced endothelial-like differentiation (Flk1+/CD34+ cells)	[163]
BM-MNCs	Density gradient centrifugation (BM)	Increased by hindlimb ischemia (DHR)	migration, adhesion	[43]
HPCs	Lin- (BM)	Increased by adrenergic treatment in culture (DCFDA)	Inhibit proliferation	[185]
EPCs or BM-MNCs	CD34+/CD117(cKit)+ (Circulation)	Increased by hypertensive rat (L-012 luminescence)	mobilization from the BM into the circulation	[186]
EPCs	BM-derived EPCs (BM)	Increased by erythropoietin (Amplex Red in supernatant)	migration, proliferation, mobilization from the BM into the circulation	[67]
HSCs	Sca-1+/c-Kit+/Lin- (BM)	Increased by conditional deletion of tuberous sclerosis complex 1 (DCFDA)	loss of quiescence, rapid proliferation, apoptosis and leukemogenesis	[62]
HPCs	CD34+/CD38- (human cord blood)	Decreased by copper chelator tetraethylenepentamine (DCFDA)	expansion in culture	[187]
HSPCs	CD34+ (human cord blood)	Decreased by hypoxia in culture (DCFDA)	expansion of CD34+/CD38-	[129]
HSPCs	Sca-1+/c-Kit+/Lin- (BM)	Increased in Fancc-/- mice (DCFDA) Increased by TNFa (DCFDA)	inhibit self-renewal or proliferation, premature senescence	[188]
HSPCs	CD45+/Lin- (BM)	ROS high (DCFDA) ROS Low (DCFDA)	myeloid skewed quiescence, self-renewal, lymphoid skewed	[21]
HPCs	32Dcl3 (cell line)	Increased by interleukin-3 or erythropoietin (DCFDA)	proliferation (G1 to S transition)	[189]

HSCs	Sca-1+/c-Kit+/Lin- (BM)	Increased by conditional deletion of FoxO1/3/4 (DCFDA)	loss of quiescence and defective repopulating capacity	[20]
HSCs	Sca-1+/c-Kit+/Lin- (BM)	Increased by buthionine sulfoximine or in Atm-/- mice (DCFDA)	loss of quiescence and defective repopulating capacity	[18]
HPCs	MO7e and B1647 (megakaryocytic cell lines)	Increased by thrombopoietin, granulocyte-macrophage colony-stimulating factor, or stem cell factor (DCFDA)	glucose transport activity	[42]
HPCs	MO7e (megakaryocytic cell line)	Increased by granulocyte-macrophage colony-stimulating factor, interleukin-3, steel factor and thrombopoietin (DCFDA)	proliferation	[56]

Description of stem and/or progenitor cells is according to the original articles. HSPCs: hematopoietic stem and progenitor cells, LT-HSCs: long-term (repopulating) hematopoietic stem cells, HPCs: hematopoietic progenitor cells, EPCs: endothelial progenitor cells, BM: bone marrow, MNCs: mononuclear cells, ac-LDL: acetylated low density lipoprotein uptake, S1P: sphingosine-1-phosphate, DHE: dihydroethidium, G-CSF: granulocyte colony stimulating factor, HGF: hepatocyte colony stimulating factor, DCFDA: dichlorofluorescein diacetate, AGEs: advanced glycation end products, DHR: dihydrorhodamine, TNFa: tumor necrosis factor alpha, Fancc: Fanconi anemia proteins, particularly the complementation group C. Studies investigating leukemia or leukemic cell lines are excluded from the list.

Figure Legends

Figure 1. Brief overview of reactive oxygen species (ROS) reactions and sources. The biological effect of ROS in the cell is dependent on their amount and duration, their source and cellular localization, and type of species. SOD: Superoxide dismutase, GPx: Glutathione peroxidases, Trx-Prx: Thioredoxin-peroxiredoxin, $O_2^{\bullet-}$: superoxide anion, H_2O_2: hydrogen peroxide.

Figure 2. Situations of physiologic ROS induction in HSCs and progenitor cells. Growth factor stimulation increases ROS which act as a second messenger in growth factor-mediated redox signaling. Change in oxygen concentration, which is often associated with energy metabolic alteration, actively and passively affects ROS level. Cell status change such as from quiescent to proliferative or migrating (often referred as activated status) involve an increase in ROS and is a physiologically reversible process. By contrast, differentiation, which is normally an irreversible process (such as myeloid commitment of multipotential HSCs) is also concomitant with increased ROS. Although mechanisms are not fully elucidated, these situations which increase ROS are linked to one another. Increase in ROS is achieved by increased their generation and/or decrease in antioxidant(s). Increased ROS may further promote the processes involving the redox alteration as a feed-forward mechanism (orange arrows).

Figure 3. Cell-intrinsic and cell–extrinsic effect of ROS on HSC and progenitor function. Two major sources of ROS in HSCs and progenitor cells are NADPH oxidase (NOX) and mitochondria electron transport chain (ETC) (red arrows). NOX is localized at the plasma membrane and perhaps at the endosome. Mitochondria may release ROS. Each produced ROS can activate specific molecular target(s) to contribute to cell-intrinsic or cell-autonomous regulation of cellular function. As cell-extrinsic or non-cell-autonomous regulation of HSC or progenitor function, ROS released from NOX or passed through the plasma membrane increase ROS in the extracellular space (solid blue arrows) which may instruct HSC or progenitors by

targeting membrane or intracellular molecules and may influence extracellular matrix or soluble factors regulating HSC or progenitor function. ROS produced from other cells in the niche may affect an important cell-cell interaction regulating HSC or progenitor function. In addition, ROS in the extracellular space may regulate the cell-cell interaction in the niche support cells (dashed blue arrow).

Figure 4. The relationship between ROS levels and stem and progenitor cell fate and function in the homeostatic state. The bone marrow regulatory niches include hypoxic or normoxic (less hypoxic) niche axis. Given oxygen (O_2) is required for ROS generation, ROS level or redox status of stem or progenitor cells is correlated with O_2 availability. In hematopoietic stem cells (HSCs), especially ones in the quiescent state, oxidative metabolism is suppressed and NADPH oxidase (NOX) enzyme expressions are low, thereby ROS generation from mitochondria and NOX is limited (ROS low). During differentiation or migration of HSCs or in hematopoietic progenitor cells (HPCs), higher ROS (ROS high) are observed with increased mitochondrial ETC (electron transport chain) activities and/or NOX expressions and serve as signaling molecules to promote self-renewal (proliferation), differentiation, migration and survival, which in turn contribute to maintain hematopoiesis and immune function. Antioxidant enzymes play an important role in regulating basal level of ROS or in the cellular adaptation in response to altered ROS generation. These include catalase, Manganese superoxide dismutase, Cu-Zn superoxide dismutase, glutathione peroxidases and peroxiredoxins. On the other hand, further increase in ROS (ROS high) with imbalance between ROS generation and anti-oxidant activity often links to apoptosis, senescence, and oncogenesis or leukemogenesis caused by pathologic HSCs.

Figure 5. Signaling pathways mediated by ROS involving stem cell fate. ROS allow stem cells to shift from the quiescent state to the functional state such as differentiation and migration. ROS can promote the survival pathway, but also lead to senescence. ROS modulate the activities

of various kinases and phosphatases, which in turn activate redox-sensitive signaling cascades. Of note, many of these molecules have also been shown to regulate basal ROS levels in stem cells, suggesting that feed-forward or feed-back mechanism by which stem cells respond to redox state and oxidative stress. Please see the main article for the details.

Figure 6. Cellular components of stem and progenitor niche and potential regulation through ROS. Hematopoietic Stem and Progenitor cells (HSPCs) reside in a niche that consists of cellular and non-cellular components. Cellular components include stem or progenitor cells, stromal cells, neurons, immune cells, osteoblastic cells, osteoclast and endothelial cells as well as the progeny of stem or progenitor cells. These cellular niche components regulate stem and progenitor cells directly through cell-cell interactions or indirectly through modifying non-cellular components including secreted neurohormonal factors, growth factors and enzymes, and extracellular matrix and oxygen (O_2) or hypoxia, as well as extracellular ROS.

Figure 7. NADPH Oxidase 2 (NOX2)-derived ROS promote hematopoietic stem/progenitor cell (HSPC) expansion and mobilization in response to ischemic injury. Ischemic injury induces expansion of low oxygen (hypoxic) area, hypoxia inducible factor-1 (HIF-1) expression and Akt activation throughout the BM, in a NOX2-dependent manner. This, in turn, regulates HSPCs expansion and mobilization from BM. Hypoxia might be induced by ROS generation which consumes oxygen, especially at the sites where oxygen supply is limited, such as the bone marrow cavity. Our data also showed matrix metalloproteinases (MMPs) are regulated by NOX2-derived ROS. These ROS-hypoxia-mediated alterations of the BM microenvironment induced by inflammation or tissue injury may play an important role in regulating stem and progenitor function to promote tissue repair and neovascularization. See ref. 24 for the details.