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Enhanced Nox1 expression and oxidative stress resistance in c-kit-positive hematopoietic stem/progenitor cells

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ABSTRACT

Although stem cells are generally thought to be resistant to oxidative stress, the fact and in detail molecular mechanism are still to be clearly identified. We herein tried to understand the overall characterization of redox regulatory signaling in hematopoietic stem cells. We purified c-kit-positive hematopoietic stem/progenitor cells from the bone marrow of healthy mice, and then evaluated their redox regulatory property. Compared to the c-kit-negative matured mononuclear cells, c-kit-positive stem/progenitor cells showed lower basic levels of intracellular reactive oxygen species, faster clearance of the accumulated intracellular reactive oxygen species, and higher resistant to oxidative stress. An overall view on the gene expression profile associated with redox regulation showed to be widely differed between cell types. We confirmed that the c-kit-positive stem/progenitor cells expressed significantly higher of Nox1 and catalase, but less of lactoperoxidase than these matured mononuclear cells. Our data suggests that stem cells keep specific redox regulatory property for defensing against oxidative stress.

1. Introduction

Adult stem cells have widely been identified in different tissues/organs, and known to persist throughout the lifetime for maintaining the homeostasis of organism, including the physiological turnover and the repair/regeneration in case of damage [1]. Several instructive genetic mouse models have recently demonstrated the unique susceptibility of stem cells to perturbations in metabolic or redox homeostasis [2–4]. In contrast, many recent studies have found that these adult stem cells likely to be highly resistant to various stresses, including oxidative stress [5,6]. Considering the critical roles in maintaining the homeostasis of organism for long-term lifetime, stem cells are generally thought to be specialized in their redox regulatory signaling for defensing against endogenous and exogenous damages/stresses. However, the sensitivity and in detail molecular mechanisms on oxidative resistance of stem cells are still to be clearly identified in comparison with these matured tissue cells.

Reactive oxygen species (ROS) are generally known to play as either physiological or pathological roles in different types of cells [7]. Appropriate ROS levels are required for physiological cellular functions and maintaining genomic stability, but excess accumulation of ROS may induce oxidative stress to damage the cells [4,8,9]. It has been reported that hematopoietic stem/progenitor cells have relatively low level of endogenous ROS when compared with their mature progeny [10,11], which seems to play critical roles in maintaining the quiescence and "stemness" [12,13], because the increase of ROS level could induce the proliferation, differentiation, and maturation of stem cells [14,15]. The relatively low ROS level in stem cells likely to associate with the up-regulated expression of numerous enzymes and multiple glutathione-independent antioxidants [16]. Functional loss of redox regulatory relevant genes, such as FOXO3a and Mdm2 has been found to contribute an increased intracellular ROS levels and the self-renewal defection of hematopoietic stem cells in animal models [17,18]. However, the property on redox regulatory signaling in stem cells has not yet been well understood.

By a directly comparison on the redox regulatory property in the purified c-kit$^+$ hematopoietic stem/progenitor cells with that of the c-kit$^-$ matured mononuclear cells from bone marrow of healthy mice, we tried to uncover the overall characterization on redox regulatory signaling in stem cells.

2. Materials and methods

2.1. Animals

We used 12-week-old male C57BL/6 mice (SLC, Japan) for the present study. All experiments were approved by the Institutional

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Animal Care and Use Committee of Nagasaki University, and experiments were performed in accordance with the institutional and national guidelines.

2.2. Separation of c-kit$^+$ stem/progenitor cells and c-kit$^-$ matured mononuclear cells from bone marrow

Bone marrow cells were collected from the femur and tibia of healthy mice. The bone marrow mononuclear cells (BM-MNCs) were isolated by density gradient centrifugation [19]. The freshly collected BM-MNCs were then incubated with anti-mouse CD117 (c-kit) antibody (Miltenyi Biotec) for 30 min [19]. After washing, the c-kit$^+$ stem/progenitor cells and c-kit$^-$ matured mononuclear cells were separated by using the Magnetic Cell Sorting system (autoMACS, Miltenyi Biotec, Auburn, CA). The purity of the c-kit$^+$ cells was around 90%, and the viability was more than 99%.

2.3. Detection of intracellular ROS levels

To measure the intracellular ROS levels, the freshly purified c-kit$^+$ cells ($n = 5$) and c-kit$^-$ cells ($n = 5$) from different mice were seeded on 96-well culture plate at a density of 1×10^4 cells/well in 100 μl 1640 medium supplemented with 10% fetal bovine serum (HyClone) and incubated at 37 °C in 5% CO_2 for 12 h. Cells were then incubated with 10 μM CM-H_2DCFDA (Molecular Probes Inc.), at 37 °C for 30 min [20]. After washing, the fluorescence intensity in each well was measured by plate reader (VICTOR™ *X*3 Multilabel Plate Reader, PerkinElmer Inc., Waltham, Massachusetts, USA). We also stimulated the c-kit$^+$ cells and c-kit$^-$ cells with 100 μM H_2O_2 for 1 h, and then investigated the accumulation and clearance of ROS in cells after stimulation.

2.4. Evaluation on oxidative stress resistance

The freshly purified c-kit$^+$ cells ($n = 5$) and c-kit$^-$ cells ($n = 5$) were stimulated with 50 and 200 μM H_2O_2 for 2 h. Cells were then labeled with propidium iodide (PI) to detect the dead cells. After twice washing, apoptotic cells were also staining with ANNEXIN V-FITC Kit according to the manufacturer's instructions (BECKMAN COULTER). Quantitative flow cytometry analysis was performed using a FACSCalibur (Becton Dickinson). We analyzed the acquired data using Cell Quest software (Becton Dickinson) [19,20].

2.5. Mouse oxidative stress and antioxidant defense pathway finder RT2 profiler PCR array

Total RNA was isolated from freshly purified c-kit$^+$ cells ($n = 5$) and c-kit$^-$ cells ($n = 5$), by using RNeasy Mini Kit (Qiagen). After the generation of cDNA using RT2 First Strand Kit (SABiosciences), PCR array was done according to the manufacturer's instructions (SABiosciences), and a total of 84 genes involved in antioxidants and ROS metabolism was included in array (Supporting information Table 1). Data from 5 separated samples of c-kit$^+$ cells and c-kit$^-$ cells was calculated for the mean fold change of expression and significance by using web-based data analysis program (SABiosciences) (Supporting information Table 1).

2.6. Western blotting

The expression levels of SOD1, SOD2, and catalase were measured by Western blotting using Mini-PROTEAN® Tetra Cell and Trans-Blot® Turbo™ Transfer System (BIO-RAD) according to the instruction manual. Briefly, the total proteins purified from the c-kit$^+$ and c-kit$^-$ cells were separated by SDS–PAGE gels, and then transferred to PVDF membranes. After blocking, the membranes were incubated with primary antibodies against SOD1 (500-fold dilution, Santa Cruz Biotechnology, Inc.), SOD2 (500-fold dilution, Santa Cruz Biotechnology, Inc.), catalase (500-fold dilution, Santa Cruz Biotechnology, Inc.), or β-actin (5000-fold dilution, Sigma–Aldrich), followed by the appropriate horseradish peroxidase-conjugated secondary antibodies. The expression was visualized using an enhanced chemiluminescence detection kit, and semi-quantitative analysis was done by measuring the density of bands using ImageQuant LAS 4000mini (GE Healthcare Life Sciences).

2.7. Statistical analyses

All results are presented as the means ± SD. The statistical significance among groups was determined by one-way analysis of variance (ANOVA) followed by post hoc test, but statistical significance between two groups was done by unpaired *t*-test (Dr. SPSS II, Chicago, IL). Differences were considered significant when $p < 0.05$.

3. Results

3.1. Different intracellular ROS level and oxidative stress resistance between c-kit$^+$ and c-kit$^-$ cells

The basic intracellular ROS levels in c-kit$^+$ and c-kit$^-$ cells were measured from the freshly isolated cells after incubation at 37 °C in 5% CO_2 for 12 h. In compare with the c-kit$^-$ cells, these c-kit$^+$ stem/progenitor cells had relatively lower ROS level ($p < 0.01$, Fig. 1A). After 1 h stimulation with 100 μM H_2O_2, the intracellular ROS levels were significantly increased to a comparable levels in both c-kit$^+$ and c-kit$^-$ cells ($p < 0.01$, Fig. 1B). Although the intracellular ROS was returned to the level that close to baseline in the c-kit$^+$ stem/progenitor cells within 18 h after stimulation ($p = 0.28$ *vs.* baseline, Fig. 1B), significant accumulation of ROS was still observed in the c-kit$^-$ mononuclear cells at18 h after stimulation ($p < 0.05$ *vs.* baseline, Fig. 1B).

We also investigated the survival of cells after oxidative stress stimulation. We found that the stimulation with either 50 or 200 μM H_2O_2 significantly induced more cell death and apoptosis of c-kit$^-$ cells than that of c-kit$^+$ cells (Fig. 2), suggested a higher oxidative stress resistance of c-kit$^+$ stem/progenitor cells in compared with their mature progeny of the c-kit$^-$ mononuclear cells.

3.2. Expression of genes involved in antioxidants and ROS metabolism

By pathway focused PCR array analysis, we widely compared the expression of genes involved in antioxidants and ROS metabolism between the c-kit$^+$ and c-kit$^-$ cells (Supporting information Table 1). The top 20 genes that up- or down-regulated were presented in Fig. 3 (Fig. 3A). We noticed that some genes involved in superoxide metabolism, including *Nox1* and *Noxa1*, were up-regulated in the c-kit$^+$ stem/progenitor cells. However, the expression of genes associated other peroxidases, including the *LPO*, was down-regulated in the c-kit$^+$ stem/progenitor cells. We have also confirmed these data by Western blot analysis (Fig. 3B).

3.3. Expression of antioxidant enzymes of SOD1, SOD2, and catalase

Western blot analysis showed that the expression of antioxidant enzymes of SOD1 and SOD2 did not significantly different between the c-kit$^+$ stem/progenitor cells and the c-kit$^-$ matured mononuclear cells (Fig. 4A and B). However, the expression of catalase was significantly higher in the c-kit$^+$ cells than the c-kit$^-$ cells ($p < 0.01$, Fig. 4C).

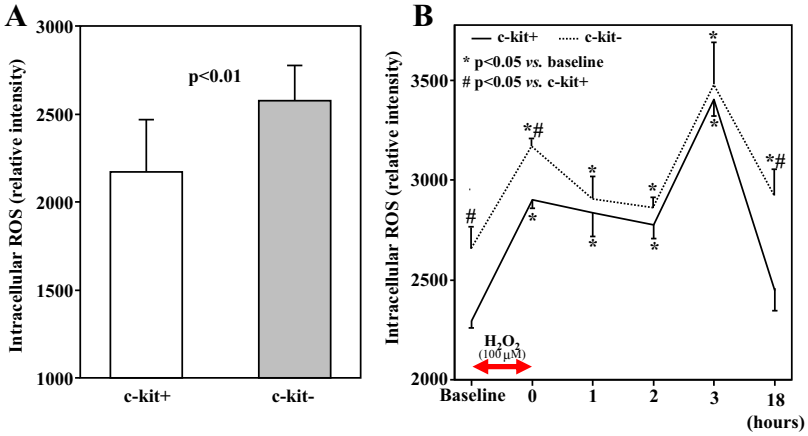

Fig. 1. ROS levels in c-kit$^+$ stem/progenitor cells and c-kit$^-$ matured mononuclear cells. (A) Cells were incubated for 12 h and then labeled with 10 μM CM-H_2DCFDA for 30 min. The ROS level was evaluated by measuring the fluorescence intensity within cells. (B) Cells were stimulated with 100 μM H_2O_2 for 1 h and then followed the changes of ROS levels at 0, 1, 2, 3, and 18 h after stimulation.

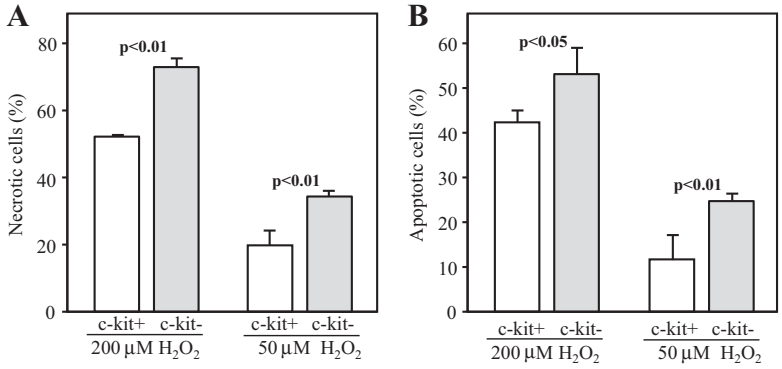

Fig. 2. Oxidative stress resistance of c-kit$^+$ stem/progenitor cells and c-kit$^-$ matured mononuclear cells. Cells were incubated with 50 and 200 μM H_2O_2 for 2 h and then stained with PI (A) and Annexin V (B) for evaluating cell death and apoptosis by flow cytometry.

A

Symbol	Fold Regulation
Nox1	10.147
Mpp4	8.151
Txnip	6.998
Ngb	4.773
Noxa1	4.708
Ptgs2	3.964
Xirp1	2.663
Il19	2.663
Nox4	2.663
Noxo1	2.608
Gpx8	-1.728
Gstk1	-1.740
Fmo2	-1.767
Sod3	-2.700
Tpo	-2.768
Rag2	-3.102
Duox1	-3.315
Prdx1	-3.756
Ehd2	-4.231
Lpo	-32.424

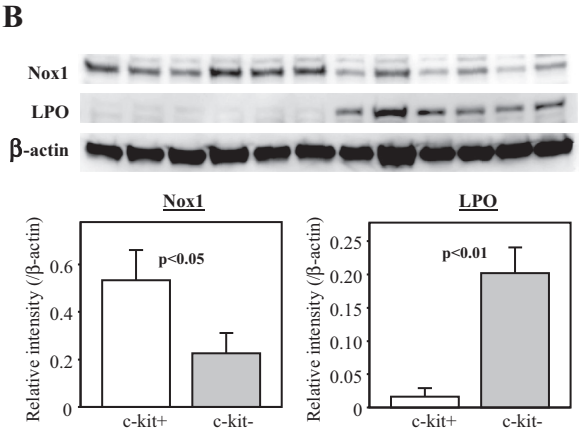

Fig. 3. The expression of genes that involved in antioxidants and ROS metabolism in c-kit$^+$ stem/progenitor cells and c-kit$^-$ matured mononuclear cells. (A) The top 20 genes that up- or down-regulated in c-kit$^+$ stem/progenitor cells. (B) Western blot analysis on the top two genes of Nox and Lpo.

4. Discussion

Although ROS can serve as critical signaling mediators and antimicrobial host defenses [7,21], the excessive accumulation of ROS is generally known to induce the cellular damage that eventually lead to aging, cardiovascular diseases, inflammation, and carcinogenesis [22]. To protect against oxidative stress-induced injuries, all mammalian have been developed powerful antioxidant

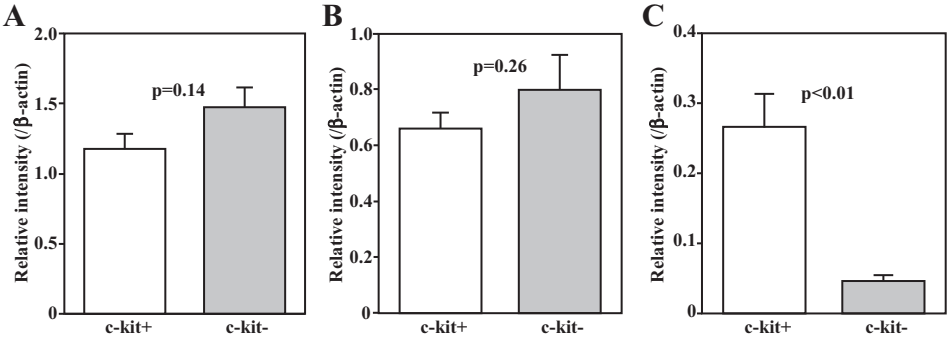

Fig. 4. Western blot analysis on SOD1, SOD2, and the catalase. Although the expression of catalase was significantly higher in c-kit$^+$ stem/progenitor cells than the c-kit$^-$ matured mononuclear cells (C), the expression of SOD1 (A) and SOD2 (B) was comparable between two cell types.

mechanisms for maintaining redox homeostasis. Therefore, the understanding on the complex network of redox regulation will be helpful for the prevention and treatment of various diseases and disorders.

As the small population tissue-specific stem/progenitor cells has been well recognized to play critical roles in carcinogenesis and the repair/regeneration of damaged tissues [1,21,23,24], redox regulation signaling in stem cells warrants specific attention in the past decade. A number of recent studies have reported the important role of redox homeostasis in regulating the self-renewal, differentiation, and genomic stability of stem cells [2–6,8–18]. It has demonstrated that moderately high H_2O_2 levels promote the differentiation of hematopoietic progenitors through JNK and FOXO activation [13,14]. We have also recently found that ROS likely plays dual roles in the genomic stability of stem cells [8], of that, a physiological level of ROS is required for effective DNA repair, but high ROS induces DNA damage. However, the characterization of redox regulatory property in stem cells has not yet been well understood.

In this study, we tried to investigate the redox regulatory property in the c-kit$^+$ stem/progenitor cells by directly comparison with the c-kit$^-$ matured mononuclear cells from the bone marrow of healthy mice. Agreed well with previous study [10], the basic ROS level in c-kit$^+$ stem/progenitor cells was detected relatively lower than the c-kit$^-$ matured mononuclear cells. Although the stimulation with H_2O_2 increased the intracellular ROS levels to a comparable levels in both of the c-kit$^+$ and c-kit$^-$ cells, these c-kit$^+$ stem/progenitor cells showed a faster clearance of accumulated ROS. This provides a reasonable explanation to the higher resistant to oxidative stress. Therefore, it would be critical to understand how the stem cells were metabolically differed from the more differentiated cells.

By screening the expression of genes involved in antioxidants and ROS metabolism, we found that the gene-expression profiling was widely varied between the c-kit$^+$ and c-kit$^-$ cells. Our data showed that the c-kit$^+$ stem/progenitor cells were highly expressed with Nox1 and Noxa1 than the c-kit$^-$ mononuclear cells. Redox homeostasis is known to be regulated by a number of oxidases, among which the plasma membrane NADPH oxidase (NOX) is recognized to be one of the major players [15]. Previous study has demonstrated that bone marrow derived hematopoietic stem cells express the catalytic subunits of Nox1, Nox2, and Nox4 isoforms of the NADPH oxidase family along with the complete battery of the regulatory subunits of Noxo1 and Noxa1, which contribute to produce constitutively low levels of ROS [25]. It has been suggested that the existence of different types of ROS-generators might provide a fine mechanism of tuning redox signaling to control the delicate balance between cell growth, proliferation, and differentiation of hematopoietic stem cells [26]. This provides reasonable explanation on the higher expression of Nox1 and Noxa1 in these c-kit$^+$ hematopoietic stem/progenitor cells than the matured c-kit$^-$ mononuclear cells.

Interestingly, some genes involved in antioxidants and ROS metabolism, such as lactoperoxidase (Lpo), was largely down-regulated in the c-kit$^+$ stem/progenitor cells. The Lpo is functionally known to play a key role in the innate immune defense by oxidizing thiocyanate into hypothiocyanite [27], which serve to kill the bacterium and fungi. Therefore, the relatively lower Lpo in the c-kit$^+$ stem/progenitor cells from bone marrow more likely due to the specificity of enhanced Lpo expression in the matured c-kit$^-$ mononuclear cells for immune defense, rather than the down-regulation of Lpo in general in stem cells.

Peroxiredoxin 1 (Prxd1), an antioxidant that well known to protect cells from metabolically produced ROS, was also down-regulated in the c-kit$^+$ stem/progenitor cells. It has recently been reported that the incubation of Prxd1 with immature bone marrow-derived dendritic cells resulted in TLR4-dependent secretion of TNF-alpha and IL-6 and dendritic cell maturation, suggest that Prxd1 may act as danger signal similar to other TLR4-binding chaperone molecules such as HSP72 [28]. Similarly, the relatively lower expression of Prxd1 in c-kit$^+$ stem/progenitor cells may also simply due to the highly expression of Prxd1 in the matured c-kit$^-$ mononuclear cells, rather than in general from the view of stem cell biology.

In conclusion, c-kit$^+$ hematopoietic stem/progenitor cells likely resistant to oxidative stress when compared with the more differentiated c-kit$^-$ mononuclear cells in bone marrow. Although widely different in the expression of genes involved in antioxidants and ROS metabolism was detected between two cell types, it is still required to understand the significance of redox regulatory property in stem cell biology.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.10.090.

References

[1] J. Pellettieri, A. Sánchez Alvarado, Cell turnover and adult tissue homeostasis: from humans to planarians, Annu. Rev. Genet. 41 (2007) 83–105.
[2] S. Chuikov, B.P. Levi, M.L. Smith, S.J. Morrison, Prdm16 promotes stem cell maintenance in multiple tissues, partly by regulating oxidative stress, Nat. Cell Biol. 12 (2010) 999–1006.

[3] Z. Tothova, R. Kollipara, B.J. Huntly, B.H. Lee, D.H. Castrillon, D.E. Cullen, E.P. McDowell, S. Lazo-Kallanian, I.R. Williams, C. Sears, S.A. Armstrong, E. Passegue, R.A. DePinho, D.G. Gilliland, FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress, Cell 128 (2007) 325–339.
[4] K. Ito, A. Hirao, F. Arai, S. Matsuoka, K. Takubo, I. Hamaguchi, K. Nomiyama, K. Hosokawa, K. Sakurada, N. Nakagata, Y. Ikeda, T.W. Mak, T. Suda, Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells, Nature 431 (2004) 997–1002.
[5] A. Valle-Prieto, P.A. Conget, Human mesenchymal stem cells efficiently manage oxidative stress, Stem Cells Dev. 19 (2010) 1885–1893, http://dx.doi.org/10.1089/scd.2010.0093.
[6] S.A. Vasilyev, M. Kubes, E. Markova, I. Belyaev, DNA damage response in CD133 + stem/progenitor cells from umbilical cord blood: low level of endogenous foci and high recruitment of 53BP1, Int. J. Radiat. Biol. 89 (2013) 301–309, http://dx.doi.org/10.3109/09553002.2013.754555.
[7] K.M. Holmström, T. Finkel, Cellular mechanisms and physiological consequences of redox-dependent signalling, Nat. Rev. Mol. Cell Biol. 15 (2014) 411–421.
[8] T.S. Li, E. Marbán, Physiological levels of reactive oxygen species are required to maintain genomic stability in stem cell, Stem Cells 28 (2010) 1178–1185.
[9] K. Naka, T. Muraguchi, T. Hoshii, A. Hirao, Regulation of reactive oxygen species and genomic stability in hematopoietic stem cells, Antioxid. Redox Signal. 10 (2008) 1883–1894, http://dx.doi.org/10.1089/ars.2008.2114.
[10] Y.Y. Jang, S.J. Sharkis, A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche, Blood 110 (2007) 3056–3063.
[11] A. Mohyeldin, T. Garzón-Muvdi, A. Quiñones-Hinojosa, Oxygen in stem cell biology: a critical component of the stem cell niche, Cell Stem Cell 7 (2010) 150–161.
[12] M.J. Kiel, S.J. Morrison, Uncertainty in the niches that maintain haematopoietic stem cells, Nat. Rev. Immunol. 8 (2008) 290–301.
[13] E. Owusu-Ansah, U. Banerjee, Reactive oxygen species prime *Drosophila* haematopoietic progenitors for differentiation, Nature 461 (2009) 537–541.
[14] A. Shinohara, Y. Imai, M. Nakagawa, T. Takahashi, M. Ichikawa, M. Kurokawa, Intracellular reactive oxygen species mark and influence the megakaryocyte-erythrocyte progenitor fate of common myeloid progenitors, Stem Cells 32 (2014) 548–557.
[15] A.S. Nadworny, M.R. Guruju, D. Poor, R.M. Doran, R.V. Sharma, M.I. Kotlikoff, R. Davisson, Nox2 and Nox4 influence neonatal c-kit(+) cardiac precursor cell status and differentiation, Am. J. Physiol. Heart Circ. Physiol. 305 (2013) 829–842.
[16] N. Kannan, L.V. Nguyen, M. Makarem, Y. Dong, K. Shih, P. Eirew, A. Raouf, J.T. Emerman, C.J. Eaves, Glutathione-dependent and -independent oxidative stress-control mechanisms distinguish normal human mammary epithelial cell subsets, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) 7789–7794.
[17] K. Miyamoto, K.Y. Araki, K. Naka, F. Arai, K. Takubo, S. Yamazaki, S. Matsuoka, T. Miyamoto, K. Ito, M. Ohmura, C. Chen, K. Hosokawa, H. Nakauchi, K. Nakayama, K.I. Nakayama, M. Harada, N. Motoyama, T. Suda, A. Hirao, Foxo3a is essential for maintenance of the hematopoietic stem cell pool, Cell Stem Cell 1 (2007) 101–112.
[18] H.A. Abbas, D.R. MacCio, S. Coskun, J.G. Jackson, A.L. Hazen, T.M. Sills, M.J. You, K.K. Hirschi, G. Lozano, Mdm2 is required for survival of hematopoietic stem cells/progenitors via dampening of ROS-induced p53 activity, Cell Stem Cell 7 (2010) 606–617.
[19] T.S. Li, S. Ikeda, M. Kubo, M. Ohshima, H. Kurazumi, Y. Takemoto, K. Ueda, K. Hamano, Diabetic impairment of C-kit + bone marrow stem cells involves the disorders of inflammatory factors, cell adhesion and extracellular matrix molecules, PLoS One 6 (2011) e25543, http://dx.doi.org/10.1371/journal.pone.0025543.
[20] T. Yoshida, S. Goto, M. Kawakatsu, Y. Urata, T.S. Li, Mitochondrial dysfunction, a probable cause of persistent oxidative stress after exposure to ionizing radiation, Free Radic. Res. 46 (2012) 147–153, http://dx.doi.org/10.3109/10715762.2011.645207.
[21] T. Finkel, Signal transduction by reactive oxygen species, J. Cell Biol. 194 (2011) 7–15.
[22] S. Saeidnia, M. Abdollahi, Toxicological and pharmacological concerns on oxidative stress and related diseases, Toxicol. Appl. Pharmacol. 273 (2013) 442–455.
[23] J.E. Visvader, Cells of origin in cancer, Nature 469 (2011) 314–322, http://dx.doi.org/10.1038/nature09781.
[24] T. Reya, S.J. Morrison, M.F. Clarke, I.L. Weissman, Stem cells, cancer, and cancer stem cells, Nature 414 (2001) 105–111, http://dx.doi.org/10.1038/35102167.
[25] C. Piccoli, A. D'Aprile, M. Ripoli, R. Scrima, L. Lecce, D. Boffoli, A. Tabilio, N. Capitanio, Bone-marrow derived hematopoietic stem/progenitor cells express multiple isoforms of NADPH oxidase and produce constitutively reactive oxygen species, Biochem. Biophys. Res. Commun. 353 (2007) 965–972.
[26] J. Boonstra, J.A. Post, Molecular events associated with reactive oxygen species and cell cycle progression in mammalian cells, Gene 337 (2004) 1–13.
[27] C. Wijkstrom-Frei, S. El-Chemaly, R. Ali-Rachedi, C. Gerson, M.A. Cobas, R. Forteza, M. Salathe, G.E. Conner, Lactoperoxidase and human airway host defense, Am. J. Respir. Cell Mol. Biol. 29 (2003) 206–212.
[28] J.R. Riddell, X.-Y. Wang, H. Minderman, S.O. Gollnick, Peroxiredoxin 1 stimulates secretion of proinflammatory cytokines by binding to TLR4, J. Immunol. 184 (2010) 1022–1030.