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## Forum Editorial

# Redox Regulation of Stem and Progenitor Cells

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**Abstract**

The field of stem and progenitor cell biology is expanding. Much of the enthusiasm is based on the potential of using stem and progenitor cells as a cellular therapy for the treatment of human disease. Although the concept of using human embryonic stem cells for therapeutic indications is intriguing, significant challenges face investigators pursuing research in this area. Therefore, renewed scientific energy is focusing on the molecular pathways that differentiate a pluripotent embryonic stem cell from more-committed tissue-specific cells. Molecular mechanisms that govern tissue-specific stem and progenitor cell function are also topics of intense investigation, given that altered function of these cells may promote a variety of human pathologies including aging, vascular disease, and cancer. Considerable progress has been made, but a clear identification of the molecular signatures of stem and progenitor cells remains elusive. A growing body of literature demonstrates that distinct functional characteristics of stem and progenitor cells are under redox regulation. In this Forum Issue, evidence for redox regulation of tissue-specific stem and progenitor cells involved in hematopoiesis and vasculogenesis/angiogenesis is presented. *Antioxid. Redox Signal.* 10, 1849–1852.

### Overview of Basic Stem and Progenitor Cell Biology

Stem and progenitor cell biology continues to expand across multiple clinical and scientific disciplines. Much of the enthusiasm is based on the potential promise of using stem and progenitor cells for the treatment of human disease. Although the concept of using human embryonic stem cells for therapeutic indications is intriguing, significant challenges face investigators pursuing research in this area, including important ethical considerations, embryonic stem cell availability, and financial support for embryonic stem cell research. Therefore, renewed scientific energy is focusing on the molecular pathways that differentiate a pluripotent embryonic stem cell, which is capable of making all cell lineages in an organism, from more-committed tissue-specific cells. The power of this experimental approach is supported by recent studies from Yamanaka and colleagues (1, 22). These elegant studies demonstrate that skin fibroblast cells, and most recently adult hepatocytes and stomach epithelial cells, can be reprogrammed to become pluripotent cells with embryonic stem cell characteristics. This remarkable achievement in stem cell biology emphasizes the importance of understanding the molecular determinants that regulate stem cell behavior.

Molecular mechanisms that govern tissue-specific stem and progenitor cell function are also topics of intense investigation, given that altered function of these cells may promote a variety of human pathologies including aging, vascular disease, and cancer (7, 21). To fulfill the strict definitions of a tissue-specific stem cell, a cell must exhibit two critical qualities. First, stem cells are required to differentiate into all the specialized cells that compose a specific tissue or organ, including progenitor cells. Second, stem cells must exhibit self-renewal potential, which is the ability to divide and make a new stem cell. In contrast, progenitor cells are more committed, with limited differentiation potential

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and self-renewal capacity. In adults, the majority of stem cells are quiescent at any one time, unless called on to replenish differentiated cells for tissue maintenance or during times of stress when an increased demand for or loss of more-differentiated cells may occur. Conversely, progenitor cells turn over more frequently and exhibit robust proliferative potential with the capacity to respond quickly to environmental stimuli that signal when an acute expansion of the differentiated cell population is needed. Although considerable progress has been made (12, 20), a clear identification of the molecular signatures for tissue-specific stem cells and distinct progenitor cell populations remains elusive. A growing body of literature demonstrates that distinct functional characteristics of stem and progenitor cells are under redox regulation. In this Forum Issue, evidence for redox regulation of tissue-specific stem and progenitor cells involved in hematopoiesis and vasculogenesis/angiogenesis is presented.

## Hematopoiesis as a Prototype for Studying Redox Regulation of Stem and Progenitor Cells

The hematopoietic system lends itself to the evaluation of stem and progenitor cell biology for several reasons, including the relative ease of hematopoietic cell isolation, the ability to replace completely the hematopoietic system of irradiated animal and human recipients, and well-established, universally accepted experimental techniques to assess hematopoietic stem and progenitor cell functions (13, 19). A recent review outlines strong rationale for using the hematopoietic system as a model for the evaluation of other tissue-specific stem cells (18). Hematopoietic stem cells are characterized by their ability to reconstitute all blood cell lineages after transplantation into lethally irradiated recipients. Experimentally, hematopoietic stem cell self-renewal capacity is examined by serial transplant studies, which require a limited number of stem cells to divide and sustain normal hematopoiesis in subsequent transplant recipients. Clinically, hematopoietic stem cell transplantation is routinely conducted as a life-saving therapy for numerous diseases, with the list of diseases continually expanding. Additionally, alterations in hematopoietic stem or progenitor cell function or both are responsible for several human diseases, including leukemia, aplastic anemia, myelodysplasia, polycythemia vera, paroxysmal nocturnal hemoglobinuria, and congenital neutropenias. Collectively, the success of hematopoietic stem cell transplantation together with hematopoietic stem/progenitor cell dysfunction being associated with significant human diseases serves as a catalyst for scientists world-wide to elucidate the cellular and molecular mechanisms underlying hematopoietic stem and progenitor cell function.

Significant advances in understanding the molecular determinants of hematopoietic stem and progenitor cell function as well as blood cell–lineage specification have been made in the area of hematopoiesis (9, 17, 18). The first evidence that redox control may regulate human hematopoiesis, specifically hematopoietic stem cell self-renewal, demonstrated that human hematopoietic stem cells could be expanded *in vitro* after culturing in hypoxic conditions (5). Since this report and reviewed in this Forum Issue, enhanced intracellular reactive oxygen species in hematopoietic stem cells have been shown to reduce hematopoietic stem cell self-renewal, to enhance hematopoietic stem and progenitor cell cycling, to increase hematopoietic stem cell senescence, and to reduce hematopoietic stem and progenitor cell survival (9, 17). Furthermore, in this issue, compelling evidence demonstrates that increased reactive oxygen species in hematopoietic stem and progenitor cells are linked to accumulation of genomic damage (6), which may increase the risk for aging alterations of the hematopoietic system and clonal hematopoietic disorders, including leukemia. Moreover, hematopoietic cells are not exposed equally to oxidant stress. An excellent example reviewed in this issue is erythroid progenitors and red blood cells, which carry molecular oxygen to tissues and are exposed to high levels of oxidative stress, requiring a tightly regulated molecular repertoire to protect against oxidant damage (9).

Given the complex interactions between DNA-damage recognition/repair, stress response, cell-cycle control, apoptosis, and redox-regulation protein networks, it should come as no surprise that critical stem and progenitor cell functions are controlled by proteins in these classic cellular-response pathways including, but not limited to, ataxia telangectasia-mutated, Fanconi anemia type C, p38 mitogen-activated protein kinase, p16, p53, and FoxO (6, 9, 17). Although recent progress has been made in understanding redox molecular mechanisms involved in regulating hematopoietic stem and progenitor cell functions, several unanswered questions remain. For example, although increased reactive oxygen species are linked to multiple hematopoietic stem and progenitor cell derangements, the source of reactive oxygen radicals (*i.e.*, electron transport chain or enzymatic production), the mechanism for perturbed reactive oxygen species concentration (*i.e.*, overproduction or decreased detoxification), and the types of reactive oxygen species that are important factors in determining hematopoietic stem and progenitor cell fate are unknown. Inflammation clearly promotes oxidant stress, genomic instability, and leukemogenesis in Fanconi anemia (6); however, whether this is a global mechanism for the pathogenesis of other hematopoietic stem cell disorders has not been explored. Given the critical role of the bone marrow microenvironment in maintaining normal hematopoiesis, a diminished supportive capacity of microenvironmental cells may induce an oxidative stress in hematopoietic stem and progenitor cells. Ultimately, translating this information to the clinic to treat and prevent hematopoietic disorders will require elucidation of the cellular and molecular target(s) involved in promoting stem and progenitor cell dysfunction and determination of the stage at which reversible changes in stem and progenitor cell function can be detected.

## Role of Redox Regulation of Progenitor Cells in Maintaining Vascular Health and Protecting from Vascular Disease

Circulating "endothelial progenitor cells" were first identified in adult peripheral blood in 1997 (2), which is 40–50 years after studies demonstrating the existence of transplantable hematopoietic stem cells (8, 15). Therefore, the field of endothelial stem and progenitor cell biology is in its infancy compared with current knowledge pertaining to stem and progenitor cell function in the hematopoietic system. By using the strict definition stated earlier, an endothelial stem

cell has not been identified. Nonetheless, scientific inquiry into endothelial progenitor cell biology is growing rapidly. A major contributing factor for this enthusiasm results from data showing that endothelial progenitor cell numbers inversely correlate with cardiovascular disease risk (10, 23, 24). These observations led to speculations that endothelial progenitor cells have a critical role in maintaining vascular homeostasis and in promoting vascular repair. In addition, investigators hypothesize that endothelial progenitor cells could serve as a biomarker for vascular disease risk before the onset of clinical symptoms and that isolation and transplantation of endothelial progenitor cells may be used as a cellular therapy.

Unfortunately, the methods used to define and isolate endothelial progenitor cells are not consistent, which has led to some controversy and is reviewed in this issue (3). Recent studies show that the majority of previous reports studying "endothelial progenitor cells" examined cells that are derived from hematopoietic stem cells and are not capable of forming blood vessels *de novo* (4, 25). These hematopoietic progenitors have also been called colony-forming unit–endothelial cells (CFU-ECs), CFU-Hill, and circulating angiogenic cells. Although these cells are hematopoietic progenitors and not endothelial progenitors, they do facilitate vasculogenesis/angiogenesis, and circulating levels inversely correlate with cardiovascular disease risk (10, 23, 24). Therefore, it is clear that these circulating hematopoietic progenitors have a critical role in maintaining vascular health. Interestingly, a distinct circulating cell population can be isolated from cord blood and adult peripheral blood; it fits the criteria for endothelial progenitor cells (11). Future studies examining the interaction between these distinct progenitor populations will be required to understand the complexity of vascular homeostasis, vascular disease pathogenesis, and the molecular basis of vascular disease. Thus, it is imperative for the vascular biology field to define carefully the origin and function of progenitor cell populations involved in vasculogenesis and angiogenesis. In addition, it will be necessary to delineate stringent experimental methods to examine distinct stem and progenitor cell functions, similar to the paradigm established for hematopoiesis, before significant strides can be made in dissecting molecular mechanisms responsible for dysfunction of these stem and progenitor cells.

Although the definition of endothelial progenitor cells has been in flux, a consensus suggests that oxidative stress and aberrant redox regulation participate in cardiovascular disease pathogenesis (3). Improvements in modalities to treat individuals with vascular disease will require elucidation of the cellular and molecular mechanisms controlled by redox changes. A clinically relevant example in this issue demonstrates that hyperbaric oxygen therapy mobilizes progenitor cells from the bone marrow, which in turn improves diabetic wound healing (14). Because hyperbaric oxygen results in a transient hyperoxia and induces nitric oxide production in the bone marrow, these observations suggest that redox changes affect progenitor cell function. Further molecular evidence for this concept is illustrated in this issue by new studies showing that inhibition of the redox activity of the DNA-repair protein, apurinic/apyrimidinic endonuclease1, significantly impairs the ability of endothelial cells to make vessels *in vitro* (16). However, a detailed understanding of the cellular and molecular events controlled by redox changes during vasculogenesis and angiogenesis is lacking.

Admittedly, much remains to be learned regarding the role of stem and progenitor cells in the processes of vasculogenesis and angiogenesis. Clearer definitions of the types of progenitors being examined will facilitate these in-depth mechanistic studies. Lessons can be gleaned from studies in hematopoiesis in which consistent definitions are used to describe stem and progenitor cells. In addition, well-defined experimental methods to evaluate stem and progenitor cell functions are critical to advance further the understanding of endothelial stem and progenitor cells in the maintenance of the vascular system.

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