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# Forum Review Article

# Redox Regulation and Its Emerging Roles in Stem Cells and Stem-Like Cancer Cells

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**Abstract**

The existence of cancer stem cells has impelled the pursuit to understanding and characterizing this subset of cells, which are thought to be responsible for tumor recurrence and to contribute to therapy resistance. Recent studies suggest that cancer stem cells seem to possess properties similar to those of normal stem cells, revealing a possible therapeutic strategy/target. For this to be feasible, it is imperative to understand the relation between cancer cells, cancer stem cells, and normal stem cells. Cancer cells have been found to be in a state of redox imbalance, an alteration in the homeostasis between oxidants and antioxidants, resulting in increased oxidants within the cell. Studies have shown redox balance plays an important role in the maintenance of stem cell self-renewal and in differentiation. Very little is known about the redox status in cancer stem cells. In this review, we focus on the sites of oxidant generation and the regulation of redox status in cancer cells and stem cells. In addition, evidence that supports the involvement of redox homeostasis for stem cell self-renewal, differentiation, and survival are reviewed. Given the significance of redox in stem cells, we also discuss the possibility of exploiting the redox status in cancer stem cells as a novel therapeutic strategy. *Antioxid. Redox Signal.* 11, 1107–1122.

## Introduction

Stem cells are characterized by having the capacity for self-renewal and the ability to differentiate to one or more different cell type(s) (100). Much effort has been devoted to the identification and characterization of cancer stem cells (CSCs), which resulted in the discovery of leukemic, brain, breast, pancreatic, and colon cancer stem cells (2, 60, 88, 93, 96). The hypothesis that recurrence and nonresponsiveness to various therapies due to CSCs has led to a surge in trying to understand the properties with which these cells are able to overcome such onslaughts. Many cancer cells possess a plethora of mechanisms used to resist chemo/radiotherapy, which include but are not limited to alterations in ABC transporters and drug metabolism, elevated repair capacity, resistance to apoptosis, and increased survival signals (89). In addition to these mechanisms, many cancer cells also show an increased ability to withstand oxidative stress and are in a state of redox imbalance.

Studies of oxidants playing a role in human disease development expanded after Denham Harman presented his "free radical theory of aging" in the 1950s. Harman suggested that free radicals produced during aerobic respiration result in genetic mutagenesis, cancer, and cell death (35). Likewise, the milestone study to identify the antioxidant enzyme superoxide dismutase (SOD) from bovine erythrocytes (by McCords and Fridovich in 1969) invoked study of the mechanisms of cellular oxidant generation and the subsequent antioxidant defense (68, 117).

In this review, we use the term "redox imbalance" to describe alterations in antioxidant-to-oxidant homeostasis, resulting in increased levels of reactive oxygen (ROS) and reactive nitrogen (RNS) species (Fig. 1). Basal, detectable levels of ROS/RNS include superoxide ($O_2^{-}\cdot$), hydrogen peroxide ($H_2O_2$), hydroxyl radical ($\cdot OH$), nitric oxide ($NO\cdot$), and peroxynitrite ($^{-}ONOO$). Levels of the oxidants are tightly regulated by antioxidants and redox-sensitive factors. Given the multifunctionality of ROS, acting as signaling molecules

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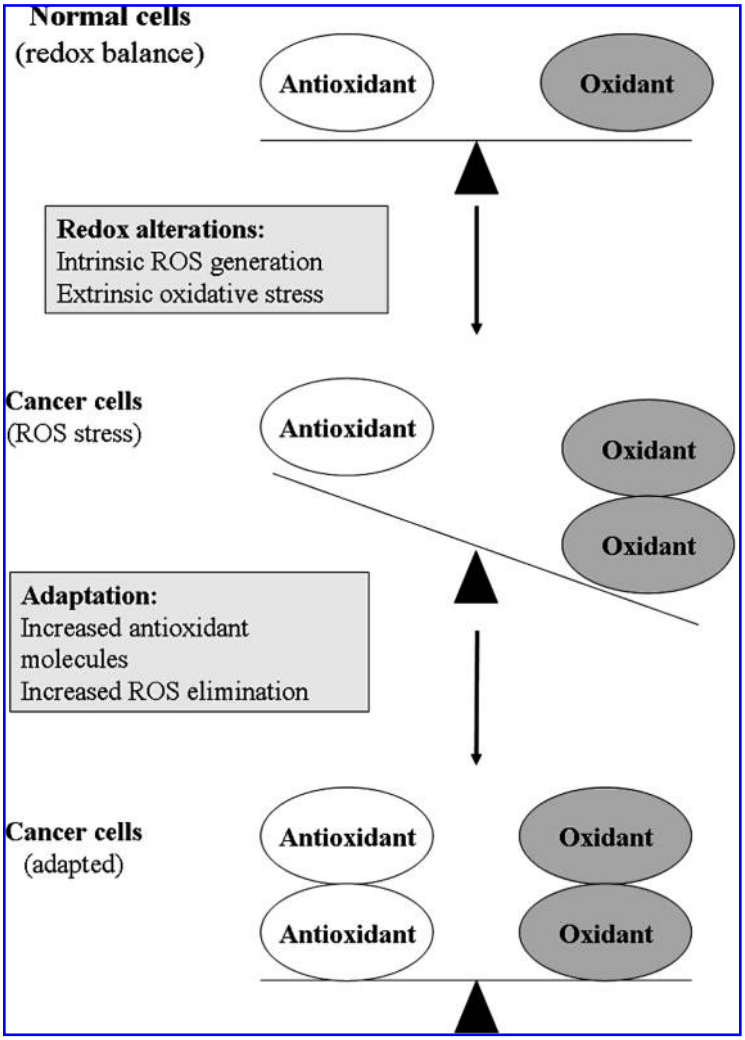


**FIG. 1. Redox imbalance in cancer cells.** Normal cells maintain redox homeostasis through mechanisms that balance oxidants by antioxidants. Cancer cells that have deregulated and dysfunctional ROS generation may exhibit increased oxidant levels compared with normal cells. Such ROS stress may trigger redox adaptation mechanisms in which compensatory increase in antioxidants may occur to establish a new redox homeostasis. In this case, both ROS generation and elimination are highly active but a dynamic balance is maintained in the well-adapted cancer cells.

but also as oxidizing, damaging molecules, quantity control within a cell is critical. Early studies on radiation, chemical carcinogens, and viruses in combination with oncogenes and tumor-suppressor models strongly suggested that abnormal accumulation of oxidants plays an important role in cancer initiation and progression (52). Increased ROS production or accumulation or both provide a favorable tumorigenic environment, for example, by perpetuating oxidatively damaged DNA. Such damage, if not repaired, may result in DNA mutation–induced activation of oncogenes or tumor-suppressor loss or both (52). In addition, redox imbalance alters fundamental regulators such as transcription factors and kinases, thus inducing structural and functional alterations that aid in malignant transformation (1).

A hallmark of highly proliferating cancer cells is enhanced aerobic glycolysis (also known as the Warburg effect). Redox imbalance may mediate this metabolic alteration either directly or indirectly through regulation of glycolytic enzymes (19). Similarly, many other important pathways altered in cancers can be modulated by ROS.

Efforts to understand further the origin and biologic characteristics of CSCs have revealed certain unique properties that are different from the "regular" cancer cells of the tumor bulk. The abilities to engage in long-term self-renewal, to differentiate into downstream progenies, and to resist drug treatment are thought to be key properties of stemlike cancer cells. It is now a question as to how one may eliminate these CSCs to "cure" an individual with this disease. It has been proposed to target the self-renewal and differentiation pathways of these CSCs (65, 121). As in terminally differentiated cells and cancer cells, redox balance has a significant role in normal stem cell biology. Understanding redox control in stem cells and cancer cells may perhaps provide insights into the redox biology of CSCs and development of a therapeutic strategy.

Studies have shown the importance of the stem-cell niche for the maintenance of "stemness." In particular, hypoxia, a state of low oxygen, has been shown to influence stem cell self-renewal and differentiation (95). With a Hoechst 33342 dye (Ho) perfusion assay, hematopoietic stem cells (HSCs) were fractionated, based on Ho dye perfusion, and analyzed for hematopoietic activity. The fluorescent dye has been used as a marker of perfusion in tissues, and in solid tumors with relation to hypoxia. From both *in vitro* and *in vivo* studies, the highest percentage of HSCs was found to be in fractions that had the lowest Ho dye perfusion, implying their localization in the bone marrow with low amounts of oxygen (79). A study correlating a hypoxic environment to reduced ROS levels was conducted by Fan *et al.*, which showed increasing ROS, *via* dichlorofluorescein (DCF) staining, of $CD34^{+}$ hematopoietic

stem and progenitor cells (HPSCs) at increasing oxygen percentages (26). Further studies that implicate the role of ROS in the regulation of maintenance of stem cells are discussed in subsequent sections.

### ROS Generation and Redox Regulation

Various sites of ROS/RNS generation within a cell are tightly regulated by site-specific and global mechanisms. Whether the oxidants serve as a functional entity or are a result of a metabolic process, often the control of oxidants has been altered in cancer cells (Fig. 2). Therefore, it is important to understand the primary ROS-generation sites in both cancer and stem cells.

### Role of Mitochondria

#### *Mitochondrial oxidant generation in cancer*

A long history of clinical and laboratory research revealed various biologic alterations in mitochondria and metabolism between normal and cancer cells. As early as the 1920s, mitochondrial dysfunction was found in various cancers, evidenced by alterations in oxidative phosphorylation capacity, impaired mitochondrial respiration, and enhanced aerobic glycolysis (113, 114). Mitochondrial dysfunction in cancer cells may result from various genetic and environmental factors, such as expression of oncogenes, loss of tumor-suppressor genes, carcinogens, aging, and hypoxia (19). Characteristics of dysfunctional mitochondria include morphologic changes, deletions/mutations of mitochondrial DNA, and altered respiratory function.

Mitochondria are essential organelles that generate cellular energy in the form of adenosine triphosphate (ATP) by metabolizing sugars, fats, and amino acids. This process is associated with the electron-transport and oxidative phosphorylation system, also known as the mitochondrial respiratory chain, and is believed to be the major cellular ROS generation site (108). The mitochondrial respiratory chain consists of five multimeric protein complexes: reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase–ubiquinone oxidoreductase (complex I), succinate dehydrogenase–ubiquinone oxidoreductase (complex II), ubiquinone–cytochrome *c* oxidoreductase (complex III), cytochrome *c* oxidase (complex IV), and ATP synthase (complex V). Among these complexes, two important electron carriers, ubiquinone (coenzyme $Q_{10}$) and cytochrome *c*, are required for electron transport. Given the reducing mitochondrial matrix environment and many single-electron reactions involved in the respiratory chain, superoxide ($O_2^{-}\cdot$) is generated through the reduction of molecular oxygen by electron leakage from various substrate-site components. This includes the electron carriers of the respiratory chain complexes I, II, and III, including flavoproteins, Fe-S clusters, and semiquinones (108).

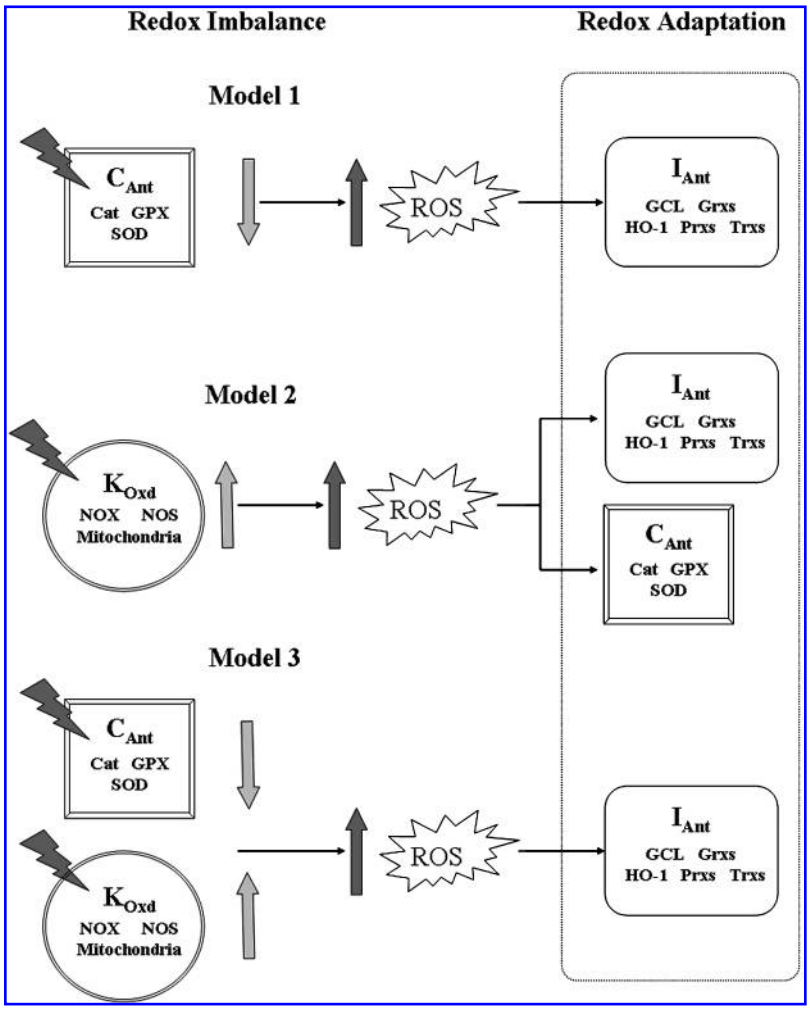


**FIG. 2. Schematic illustration of possible models for redox imbalance and adaptation.** Model 1: A reduction of constitutively expressed antioxidants ($C_{Ant}$) such as catalase (Cat), glutathione peroxidases (GPX), and superoxide dismutase (SOD) can lead to accumulation of ROS, which in turn induce the expression of inducible antioxidants ($I_{Ant}$) such as γ-glutamyl-cysteine synthetase (GCL), glutaredoxins (Grxs), heme oxygenase-1 (HO-1), peroxidredoxins (Prxs), and thioredoxins (Trxs). Model 2: Aberrant increase in the activity of oxidant generators ($K_{Oxd}$) such as the mitochondria, NAD(P)H oxidase (NOX), and nitric oxide synthase (NOS) may result in an elevation of ROS, which in turn triggers both $I_{Ant}$ and $C_{Ant}$ as adaptive responses. Model 3: Both decrease in $C_{Ant}$ expression and increase in $K_{Oxd}$ can lead to substantial increase in ROS, leading to elevated expression of $I_{Ant}$.

Increased electron leakage and enhanced generation of mitochondrial $O_2^{-}\cdot$ and its byproducts are common consequences of mitochondrial dysfunction. Further conversion of elevated $O_2^{-}\cdot$ may increase mitochondrial hydrogen peroxide ($H_2O_2$) and hydroxyl free radical (·OH) levels. Besides respiratory chain electron leakage-induced ROS generation, a recent study showed several enzymatic sources contributing to mitochondrial oxidant generation in cancer cells. These enzymes included the mitochondrial membrane-bound oxidase, dehydrogenase, and other mitochondria-specific proteins such as mitochondrial glycerophosphate dehydrogenase (mGPDH) (21).

### *Mitochondria in stem cells*

Stem cells that undergo differentiation are in a dynamic state in which organelles such as the mitochondria undergo major changes (23). One can surmise that this process of change initiates a cascade of events that further influences differentiation to their terminal target cells. For example, electron micrograph (EM) evaluation revealed fewer mitochondria in embryonic stem cells, with smaller size and fewer cristae (20, 76). On differentiation, mitochondria mass and mitochondrial DNA number were found to increase (20, 22, 92). Consequently, increases in DCF-DA staining, representative of ROS generation, and ATP generation also was seen (20). Development of mitochondria results in the use of oxidative phosphorylation, a more efficient ATP-generating process, which also results in an increase in ROS generation. Furthermore, this switch to mitochondrial respiration appears to be required by some cells for further differentiation. Chung *et al*. (22) found that on embryonic stem cell differentiation, cells required a metabolic switch from anaerobic glycolysis to mitochondrial respiration for proper cardiomyogenesis and function (22). Gene-expression analysis attributed the metabolic switch to increases in mitochondrial respiratory chain and Krebs' cycle–associated genes and modulation of glycolytic enzymes.

## Role of Endoplasmic Reticulum

### *Endoplasmic reticulum oxidant generation in cancer*

The endoplasmic reticulum (ER) is the major site of protein folding and maturation (*i.e*., protein disulfide bond formation) through an oxidizing environment that favors this process. ER oxidoreductases, such as PDI, a thioredoxin-like protein, are the key components involved in the oxidation, reduction, and isomerization of proteins during disulfide formation (107). Redox regulation of PDI has been found to be associated with ER-dependent oxidant generation, which has been estimated to account for a significant portion of ROS generation in mammalian cells (107). However, the exact ratio and underlying mechanism have not been well explored in cancer cells. Two biomarkers of ER stress, glucose-regulated protein-78 (GRP78) and glucose-regulated protein-94 (GRP94), are strongly induced in different tumors and suggest a correlation of ER stress and tumorigenesis (56). Deregulation of protein disulfide isomerase (PDI)-induced high oxidation rate or protein misfolding in the ER may trigger the unfolded protein response (UPR) and promote cellular oxidant generation. Human ER oxidoreductin 1–like proteins (ERO1-Ls) specifically bind PDI and induce oxidation of the PDI active site [-C-X-X-C-]. Oxidized PDI further oxidizes the substrate protein for formation of a disulfide bond. During this process, ERO1-L is reoxidized by transferring an electron to molecular oxygen from its thiol groups, thus generating superoxide byproducts. The two isoforms of ERO1-L are ERO1-L$\alpha$ and ERO1-L$\beta$, which are controlled under different induction mechanisms. Hypoxia induces ERO1-L$\alpha$, whereas the ER protein misfolding-mediated unfolded protein response (UPR) promotes ERO1-L$\beta$.

Crosstalk between the ER and other organelles also contributes to cellular redox alterations. Impairment of mitochondria oxidative phosphorylation capacity by ER stress–induced calcium release may promote mitochondrial oxidant generation. ER stress also may enhance lysosome activation, lysosomal-oxidant generation, and induce autophagy, a controlled self-degradation process involved in cell death and survival. Study of an ER-stress inducer, thapsigargin (binds sarco/endoplasmic reticulum $Ca^{2+}$-ATPases), demonstrated that activation of inducible nitric oxide synthase (iNOS) is a mediator of ER stress–induced oxidative stress in mouse NIH3T3 cells (40).

### *The endoplasmic reticulum in stem cells*

Morphologic comparison between undifferentiated and differentiated hESCs by electron micrography (EM) showed little to unobservable ER formation (76). To understand further the role of ER proteins, GRP78$^{+/-}$ and GRP78$^{-/-}$ mice have been generated. GRP78$^{-/-}$ mice were embryonic lethal, attributed to their requirement for proliferation and prevention of apoptosis (63).

## Cell-Surface Oxidant Generation in Cancer

### *NAD(P)H oxidases and dual oxidases*

NAD(P)H oxidase (NOX) and dual oxidase (DUOX), a family of flavoproteins commonly localized to the cell plasma membrane, generate $O_2^{-}\cdot$ by the transfer of a single electron to molecular oxygen during oxidation of NAD(P)H. Further $O_2^{-}\cdot$ generation may be promoted by growth factors, cytokines, and calcium signals. $O_2^{-}\cdot$ in the cytosol can be converted to $H_2O_2$ by SOD1. NOX/DUOX-mediated ROS have been shown to function as a cellular signaling messenger to regulate cell metabolism, proliferation, and growth in human cells (54). Seven isoforms of NOX/DUOX have been identified in human tissues, including NOX1 to 5 and DUOX 1 and 2. The different isoforms of NOX/DUOX have been shown to have different induction mechanisms and tissue-specific localizations (55). Recently, specific NOXs, such as NOX4, have been found to be highly active and required for oncogenic signal transduction in pancreatic cancer cells (70).

### *NOX in stem cells*

The NOX family members and their roles have been investigated in stem cells. NOX1, NOX2, and NOX4, along with their regulatory subunits (p22, p40, p47, p67, rac1, rac2, NOXO1, and NOXAI), have been found to be expressed both at mRNA and protein levels in human hematopoietic stem/progenitor cells (HSPCs) (83). ROS levels were reduced with the use of the nonspecific flavin inhibitor DPI and NOX-specific inhibitor, apocynin (83). It was found that HSPCs have constitutive ROS generation but were minimally stim-

ulated when treated with phorbol myristate acetate (PMA) or granulocyte colony-stimulating factor (G-CSF) (83). Differentiation of mES cells in embryoid bodies induced NOX1, 2, and 4 mRNA expression in varying levels, with NOX4 having the most constant expression (10). Interestingly, treatment of the embryoid bodies with $H_2O_2$ stimulated further mRNA expression of NOX1 and NOX4 (10).

### Nitric Oxide (NO)

#### *NO generation in cancer*

Nitric oxide synthases (NOSs) are responsible for the generation of nitric oxide (NO), the major RNS. Various NOS isoforms include endothelial (eNOS), inducible (iNOS), neuronal (nNOS) and mitochondrial (mtNOS) nitric oxide synthases. NO generation is tightly regulated by NOSs and is responsible for a variety of signaling processes. The mitochondrial nitric oxide synthase (mtNOS), a unique isoform of NOS (a splice variant $\alpha$ of neuronal nitric oxide synthase isoform, nNOS-$\alpha$), has been discovered to be located on the inner membrane of the mitochondria and to release NO into the mitochondrial matrix (30). mtNOS-induced NO formation may reversibly inhibit the mitochondrial respiratory chain, further triggering $O_2^{-}\cdot$ generation and increased $[O_2]_{0.5}$ (the concentration of $O_2$ that sustains 50% of the maximal respiratory rate) (3). Reactions between NO and $O_2^{-}\cdot$ results in generating the highly reactive radical, peroxynitrite ($ONOO^{-}$), which may cause oxidation of cellular proteins (24). A recent investigation observed increased mtNOS activity by 46% in a human colorectal cancer progression study (90). In addition, a highly significant relation between mitochondria mass and endogenous NO in leukemia cells was found (13). Primary chronic lymphocytic leukemia (CLL) cells showed greater mitochondria mass compared with normal lymphocytes, in which elevated mRNA expression of mitochondria biogenesis factors NRF-1, PGC-1, PRC, and TFAM was seen to be highest in samples with greater NO levels. Further studies on cultured B cells with the NO donor SNAP stimulated mitochondria generation, whereas use of the NO synthase inhibitor L-NMMA reduced mitochondrial mass (13).

#### *NO in stem cells*

The role of nitric oxide in inducing differentiation, particularly into cardiac myocytes from human and murine embryonic cells, revealed dynamic gene-expression changes. In murine embryonic cells, the mRNA levels for SGC$\alpha$ 1, the receptor for NO genes, increased from undetectable in undifferentiated cells to substantially higher levels (up to 60-fold) after 10 days of differentiation (53). Similarly, SGC$\beta$ 1 was found at low levels in undifferentiated cells but also increased throughout the 10-day differentiation period, although not as much as SGC$\alpha$ 1 (53). All three NOS isoforms also were detected in varying levels, with NOS-1 highest in undifferentiated cells and decreasing significantly on differentiation. Conversely, NOS-2 and NOS-3 levels were highest after 10 and 7 days of differentiation, respectively. Further studies by the same group in human embryonic stem cells found both SGC$\alpha$ 1 and SGC$\alpha$ 2 to increase on differentiation, whereas an increase in SGC$\beta$ 1 but decrease in SGC$\beta$ 2 was observed (71). In contrast to mES cells, NOS-1 mRNA levels increased about threefold after 8 days of differentiation before decreasing below the level measured in undifferentiated cells. NOS-2 mRNA levels peaked at day 14 before decreasing, whereas NOS-3 peaked at day 20. These dynamic changes in both the NOS and SGC isoforms reflect the role NO signaling may have in differentiation of hES cells, which may also vary from mES differentiation (71).

### Antioxidants and Their Alterations in Cancer and Stem Cells

Cells are equipped with a well-fortified defense system for the proper elimination of oxidants to maintain redox balance. Antioxidant levels can be regulated directly by oxidant levels through redox-sensitive transcription factors. The major antioxidants can be divided into two categories: thiol- and nonthiol-containing antioxidants.

#### *Cellular nonthiol antioxidant enzymes*

Superoxide dismutase and its expression in stem cells. SODs are essential enzymes that convert $O_2^{-}\cdot$ to hydrogen peroxide. The three isoforms are SOD1, SOD2, and SOD3, which are continually expressed in cells. SOD1 is a copper-zinc SOD, localized mainly to the cytoplasm and present in low levels in the mitochondrial intermembrane space. SOD1 is the primary enzyme that responds to $O_2^{-}\cdot$ generated from cytosolic NOX, xanthine oxidase, and the membrane-bound P-450 enzyme. SOD2 is the mitochondria-specific isoform manganese SOD and considered to be a major cellular SOD. SOD2 is localized in the mitochondrial matrix and plays a crucial role to scavenge mitochondria-generated $O_2^{-}\cdot$ and respiratory chain–derived $O_2^{-}\cdot$. SOD3 is an extracellular copper-zinc SOD and is functional mainly outside of the cell.

Alterations of SOD2 expression or activity have been demonstrated in many different cancers. Analysis of several pancreatic cancer cell lines varying from low to high SOD2 levels found that reduced expression was due to epigenetic silencing *via* methylation of the sod2 locus (43). Similar results were also found in multiple myeloma cells, and in both cases, overexpression of SOD2 increased sensitivity to 2-methoxyestrediol (2-ME)-induced DNA damage (43, 44). However, increasing evidence has shown the upregulation of SOD2, sometimes also including SOD1, in cancer metastasis and aggressive tumorigenesis (36). These varying observations may suggest the dynamic role SODs have in regulating cellular redox status in cancer, where defects in regulation of SODs might be a cause of induction of redox imbalance. Conversely, abnormal upregulation of SODs could be an adaptation to intrinsic oxidative stress conditions in cancer, which contributes to survival of cancer cells under stress.

Changes in expression of SODs have been observed in stem cells on differentiation. SOD2 in both human and murine embryonic stem cells has been found to decrease on differentiation (20, 91, 92), whereas SOD1 was observed to increase (20). Knockout models for both SODs also have been generated and investigated. To see the role of Cu/Zn-SOD (SOD1) in myocardial ischemic reperfusion injury, mice with targeted deletion of SOD1 to the heart were generated. $SOD1^{-/-}$ resulted in viable, normal-growing male and female mice (116). The $\cdot OH$ levels, as measured by 2,3-dihydroxybenzoic acid (2,3-DHBA), after ischemia/reperfusion injury, were significantly higher in $SOD1^{-/-}$ hearts compared with both wild-type and $SOD1^{+/-}$ hearts. Similarly, malonaldehyde (MDA) formation, another marker for oxidative stress and lipid

peroxidation, was significantly higher in $SOD1^{-/-}$ hearts during postischemic reperfusion. Interestingly, the authors noted no changes in activities of other antioxidants, such as Mn-SOD (SOD2), catalase, GP, GR, and glucose-6-phosphate dehydrogenase (G6PDH) in $SOD1^{+/-}$ and $SOD1^{-/-}$ hearts (116). More specifically, total knockout of SOD1 resulted in viable mice that displayed no developmental abnormalities. However, by unknown mechanisms, fertile $SOD1^{-/-}$ female mice showed significantly higher postimplantation embryonic death, suggesting the significance of SOD1 in reproductive health. SOD1 activity was not detectable in $SOD1^{-/-}$ mice (*i.e.*, brain, liver); however, minimal SOD1 activity, believed to be from extracellular SOD, was seen in the lung. Similarly, no changes in antioxidant activities in $SOD1^{-/-}$ hearts were seen. $SOD1^{-/-}$ mice displayed no difference in sensitivity to hyperoxia compared with wild-type mice but were significantly sensitive to paraquat, an $O_2^-$•-generating herbicide. The authors suggested that SOD2 may be more critical in handling hyperoxia-induced stress, as the mitochondrion is the primary site for ROS generation, and SOD1 might be more critical in survival from paraquat-induced stress (38, 42). As such, generation of homozygous null SOD2 mice die within 10 days after birth (61). It is possible, however, to culture $SOD2^{-/-}$ mouse fibroblasts, as described by Huang *et al*. (42). These results underscore differences between *in vivo* and *in vitro* conditions and the importance of considering the microenvironment for antioxidant and oxidant studies.

### *Catalase and its expression in stem cells*

Catalase is a tetrameric hydrogen peroxide–scavenging enzyme that responds mainly to peroxisomal ROS and extreme cellular oxidative stress (12). Catalase is a constitutively expressed enzyme with cytosolic and peroxisome localizations. Catalase has been found to be decreased in many cases of cancer, which may also reflect peroxisome defects (66, 75). Kahlos *et al*. (49) found the highest upregulation of catalase mRNA and activity in malignant mesothelioma cells most resistant to oxidant-generating menadione and the cytotoxic agent, epirubicin.

Catalase protein levels were found to decrease on differentiation in hESCs (20). $Catalase^{-/-}$ mice generated by gene targeting appeared phenotypically normal, with no alterations in other antioxidants such as GPX1, SOD1, and SOD2 (39). No catalase protein levels were detected, whereas minimal catalase activity was observed in the knockout mice. This observation might be due to the presence of other antioxidants capable of reacting with $H_2O_2$. Similar to SOD-knockout mice, $Cat^{-/-}$ mice, compared with wild-type and $Cat^{+/-}$ mice, showed no difference in response to hyperoxia treatment. However, both $Cat^{+/-}$ and $Cat^{-/-}$ mice brain mitochondria were found to be more susceptible to traumatic brain injury in a Controlled Cortical Impact Injury (CCII) model (39). This study reveals that catalase activity and its functional importance may depend on the tissue and localization. It would be worthwhile to measure and compare catalase expression further in various malignant tissues and also in cancer stem cells.

### *Heme oxygenase and its expression in stem cells*

Heme oxygenases (HOs) are the rate-limiting enzymes that catalyze heme degradation, yielding biliverdin, CO, and iron (48). Biliverdin is further reduced to bilirubin by BVR (biliverdin reductase). The antioxidant functions of HOs are believed to be mediated mainly by the products of biliverdin and bilirubin. HOs have been identified with two isoforms in human cells. HO-1 is an oxidant-induced stress-response protein with various cellular compartmental localizations. HO-2 is a constitutively expressed enzyme for which levels of expression depend on the cell types (48). HO-2 is localized primarily to cell-membrane structures that contain high concentrations of heme proteins. Increasing evidence indicates that HO-1 is abnormally induced or genetically upregulated to adapt to oncogenic stress. This upregulation therefore plays an important role in promoting malignant cell survival and conferring resistance to carcinogen exposure, oxidative stress, hypoxic microenvironments, and even drug treatment (48).

Investigation of HO-1 in human mesenchymal stem cells showed dynamic changes in mRNA and protein levels on neuronal differentiation (6). Undifferentiated MSCs showed higher HO-1 mRNA and protein levels as compared with cells that underwent differentiation. Interestingly, the use of hemin as an HO-1 inducer on undifferentiated MSCs was able to increase HO-1 mRNA, its activity, and resistance to glutamate-induced cytotoxicity (6). These results suggest that dynamic changes in HO-1 on neuronal differentiation of MSCs may also reflect changes in susceptibility to cytotoxic agents (6).

### *Cellular thiol-dependent antioxidants*

Glutathione system. Glutathione (GSH) is a ubiquitous small reducing thiol peptide and functions as an important intracellular redox buffer. Cellular GSH status is regulated mainly by GSH synthesis, transport, and metabolism. $\gamma$-Glutamyl-cysteine synthetase (GCL) and glutathione synthase (GS) catalyze GSH synthesis by using glutamate, cysteine and glycine as substrates (33). Glutathione-*S*-transferases (GSTs) and some membrane GSH efflux pumps mediate intracellular and extracellular GSH conjugation and transport. The catabolism of GSH is a process catalyzed mainly by $\gamma$-glutamyltranspeptidase ($\gamma$-GT). The metabolism of GSH consists of enzymatic and nonenzymatic redox reactions at the reactive thiol group (28). GSH is a scavenger for free hydroxyls, protein, and DNA radicals by nonenzymatic redox reactions. GSH can also react with NO to form a stable molecule, *S*-nitrosoglutathione (GSNO) by nonenzymatic conjugation and consequently regulates cellular RNS generation and protein *S*-glutathionylation (86). In addition, GSH may function as the substrate for the reduction of $H_2O_2$, lipid peroxides, and $OONO^-$ by specific antioxidant enzymes such as glutathione peroxidases (GPXs), phospholipid hydroperoxide GPX (PHGPXs), and peroxiredoxins (Prxs). In these metabolic processes, GSH is oxidized to GSSG, which can be recycled back to its reduced form, GSH, by glutathione reductase (GR) (28). The subcellular GSH content and GSSG/GSH ratios are important for maintaining proper cell structures and protein functions. Intracellular GSH concentrations are in the millimolar range; however, extracellular GSHs are in micromolar levels (28). Cells maintain a high GSH/GSSG ratio, pertinent for survival, in the cytosol, nuclear matrix, and mitochondria. A higher oxidized-to-reduced glutathione ratio (GSSG/GSH) ranges from 1:3 to 1:1, found in the lumen of the ER (32).

Many abnormal alterations of the GSH antioxidant system have been observed in cancer cells. GSH can function both as a

detoxifying and reducing antioxidant by conjugating to carcinogens and reducing carcinogen-generated oxidants (28). High intracellular GSH content has been shown to be an important metabolic cofactor for the development of drug resistance, antiapoptosis, and cell survival. Particularly, the activation or upregulation of GSTP (GST $\pi$ isoform), GSH efflux pumps, MRP, GCL, BCL-2 family proteins, and protein *S*-glutathionylation has been seen in malignant cells (28, 99).

Glutathione peroxidases (GPXs) are also commonly altered in cancer cells. GPXs are a family of GSH-dependent antioxidants that scavenge $H_2O_2$ in cells with acute or moderate oxidative stress. Pro198Leu polymorphisms, decreased activity, and downregulation of GPX1, the main cytosolic isoform, have been demonstrated in different cancer cells (57). In addition, GPX3 (an androgen-controlled plasma GPX) has been shown to be suppressed in prostate cancer. (118). Mitochondrial PHGPX (GPX4) is an essential molecule that protects membrane lipids from peroxidation; however, no evidence has been reported to show a strong correlation with tumorigenesis. A study by Wang *et al*. (110), with MCF-7 breast cancer cells, showed that overexpression of PhGPX enhanced removal of singlet oxygen–induced lipid peroxidation and removed initiators for free radical chain reactions.

Whereas GPX1 increased on differentiation in hESCs, GPX2 decreased in both hESCs and mESCs (20, 92). GPX3 and 4 were found to decrease in mESCs (92), revealing dynamic changes in antioxidants on differentiation. The significance of the glutathione family in development has been evaluated by using various knockout and overexpression mouse models. Generation of individual GPX knockouts has been accomplished, including $GPX1^{-/-}$ and $GPX4^{-/-}$ (37, 115). $GPX1^{-/-}$ mice were viable and, similar to SOD KO mice, did not show significant differences in response to hyperoxia (37, 39). No significant changes were found in activities of catalase, SOD1 and 2, and glucose-6-phosphate dehydrogenase (G6PDH) in various tissues (*i.e*., brain, heart, kidney, liver, and lung) of $GPX1^{-/-}$ mice compared with the wild type or $GPX1^{+/-}$. The only exception was found with glutathione reductase (GR) in the liver of $GPX1^{-/-}$, which was 18% higher compared with the wild type or $GPX1^{+/-}$ (37).

Conversely to GPX1-knockout mice, homozygous null GPX4 mice are not viable, with embryonic death by midgestation (8, 115). Histologic examination found that $GPX4^{-/-}$ embryos failed to form organized embryonic or extraembryonic cavities. $GPX4^{+/-}$ mice, however, are viable and appear normal despite having 50% reduction in GPX4 mRNA in various tissues (115). Borchert *et al*. (8) demonstrated, with an *in vitro* embryogenesis model, the role of specific isoforms of GPX4 in development. Their study found that the mitochondria- and nucleus-targeted GPX4 isoforms may play an important role in embryonic development of both the brain and heart (8). Specifically, cells with mitochondria-targeted GPX4 siRNA showed increased TUNEL staining in the hindbrain compared with controls, indicating the role mitochondrial GPX4 may have in neuron cell survival (8).

Glutathione reductase (GR), another GSH metabolism enzyme, has been reported with altered activity in certain cancer tissues. Significantly elevated GR and GPX activity was measured in patient glioblastoma tumor and transitional meningioma samples when compared with normal brain tissues (98). Conversely, varying levels of GR activity were observed between patient breast tumor samples (97). The variations observed between the tumor types and within the group indicate that redox alterations are dynamic and not always uniform. Taken together, these studies suggest that high intracellular GSH concentrations and GSH transport, synthesis, and distribution might be important cell-survival factors in response to cellular redox imbalance in cancer cells.

### Thioredoxin System

Thioredoxins (Trxs) are another group of important redox molecules that are needed to maintain cellular redox status. Intracellular Trxs concentrations are in the micromolar range, which seems to indicate a less significant role as direct ROS/RNS scavengers compared with GSH under normal physiologic conditions. However, recent studies showed that Trxs might have a more important role in controlling cellular enzyme redox status and in regulating signal-transduction cascades (84). Trxs contain dithiol groups as the reducing active sites and can be oxidized to a disulfide form. The antioxidant function of Trxs requires thioredoxin reductases (TrxRs) to convert oxidized Trxs back to the reduced form, by using NADPH as the substrate. Reduced Trxs participate as the substrate for some thioredoxin-dependent peroxiredoxins (Prxs), GPXs, and PHGPXs to reduce $H_2O_2$, lipid hydroperoxides, and oxidative stress–induced protein disulfides (7, 73). It is worth noting that the expressions of GSH- and Trx-metabolizing enzymes seem to be inversely regulated. In a multiple study with cancer, TrxR and GPX1 appeared to be regulated in an inverse manner (31).

Human cells contain several Trx and TrxRs isoforms. Trx1 and TrxR1 are the most abundant cytosolic and nuclear isoforms, whereas mitochondria-specific isoforms include Trx2, TrxR2, and TrxR3. Trx overexpression has been demonstrated to be associated with cell proliferation, antiapoptosis, invasion, and drug resistance in various cancers. A recent study suggested that nuclear elevated Trx1 might be the specific molecule that provides a link between Trxs and malignance (109). Conversely, TrxRs have not been found to be significantly altered in cancer. The role of Trxs in tumorigenesis might highly depend on the cellular status and distribution of Trxs. Upregulation of Trxs may be an important survival response in cancer cells to redox imbalance from GSH-metabolism deficiency. Trx also appears to be required for viability, as $Trx^{-/-}$ mice were found to be embryonic lethal. $Trx^{-/-}$ mice showed abnormal blastocyst development, whereas $Trx^{+/-}$ mice were fertile and viable (67).

### Peroxiredoxins, Glutaredoxins, and Sulfiredoxins

Besides catalase and GPXs, peroxiredoxins (Prxs) are another family of $H_2O_2$-scavenging enzymes known as alcohol- or water-mediated oxidant-induced antioxidants. Prxs have one or two cysteine residues as the active site(s), dependent on the isoforms. All members of this family have both peroxidase- and GSH-dependent co-substrate activity. Trxs play an important role in regulating the activation of Prxs by maintaining the molecules in a reduced state. Prxs have been found commonly upregulated in a variety of cancer cells (50). Prx1 is the main cytoplasmic isoform and has been demonstrated to be overexpressed in several cancers such as breast carcinoma, lung carcinoma, and bladder carcinoma (74, 78, 85). Recently a study also showed that other cytoplasmic isoforms, such as Prx2, 4, and 6, are upregulated in certain

tumors (51, 85). Prx3 and 5 are two mitochondria-specific isoforms that have been found to be elevated in response to increased $H_2O_2$ and lipid peroxide levels in malignant cells (51, 74, 78). Elevated expression of oxidant-induced cytosolic and mitochondrial Prxs might be an important survival mechanism to maintain redox balance in cancer cells and to avoid cell death under stress conditions.

Glutaredoxins (Grxs) function mainly as electron donors for the reduction of cellular sulfur metabolic molecules, protein glutathionylation, and disulfide formation. Grx1 is the most abundant isoform and similar to Trx1 and is involved in regulating the redox status of many proteins responsible for signal transduction. Elevated Grx1 expression has been found to be related to drug resistance in breast cancer cells (69). Grx2, the mitochondria-specific isoform, is known to have a protective role in cancer cells with oxidative stress. Recently, a study confirmed two human testis–specific isoforms of Grx2 that are abnormally expressed in various cancer cells (62). The upregulation of specific Grxs might be important cell-survival factors that regulate the activation of redox-sensitive signal-transduction pathways in response to redox imbalance in cancer cells.

Sulfiredoxin (Srx) is a newly identified antioxidant protein that regulates cellular sulfur metabolic molecules and reversible glutathionylation of overoxidized proteins such as Prxs (17). Because Srxs share similar cellular reduction–regulating functions with Trxs and Grxs, a link might exist between Srx activation, tumorigenesis, and cancer cell survival (27). However, further studies are required in this area.

It is evident that on stem cell differentiation, oxidant-generating enzymes as well as antioxidants are dynamically expressed (Fig. 3). As these processes are highly regulated, disruptions of these homeostasis mechanisms, as revealed through knockout studies, could prove to be lethal or highly susceptible to oxidants. Redox imbalance clearly influences cell differentiation, survival, and response to therapies. It is therefore important to understand how, mechanistically, ROS may exert its influence on cancer cells and stemlike cancer cells.

## Significance of Redox Homeostasis in Stem Cell Biology

### *Signaling pathways and cancer stem cells*

A number of studies have been conducted to investigate the signaling pathways important in regulation of stem cells and the role they may have in cancer stem cells. Given the similarity between normal stem cells and cancer stem cells, it is

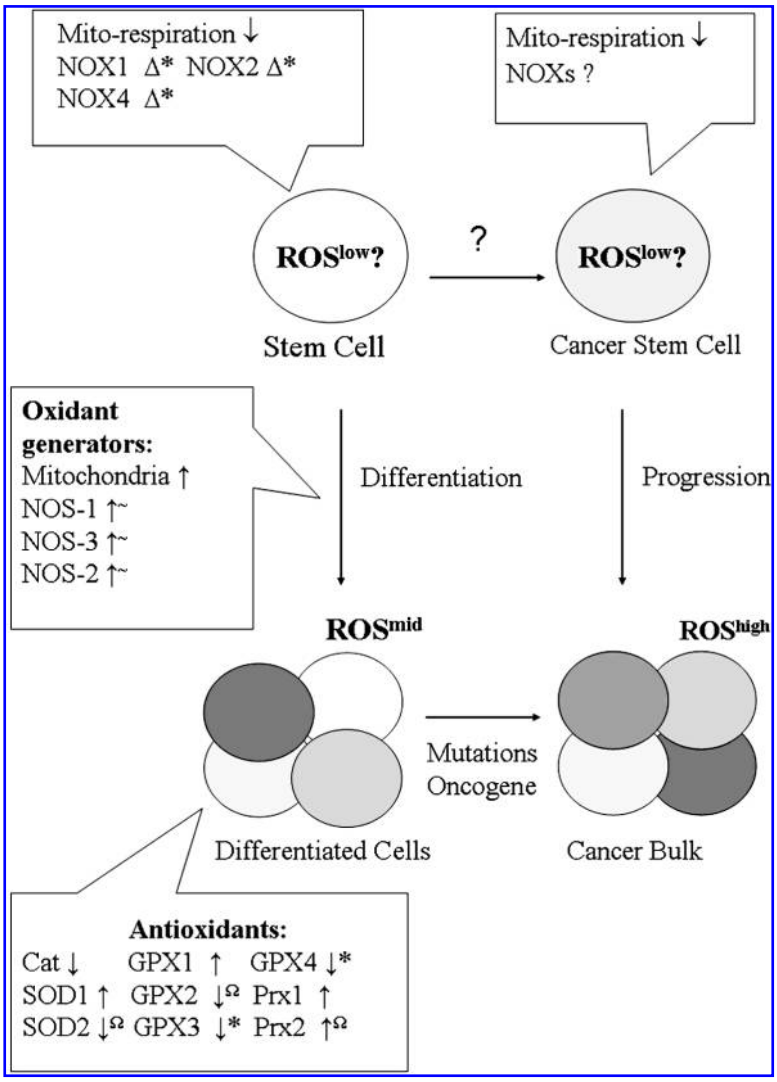


**FIG. 3. Dynamic redox alterations in stem cells and relevance to stemlike cancer cells.** Low oxidative metabolism and under developed mitochondria are observed in stem cells. This is associated with low ROS ($ROS^{low}$) generation. Basal expression of ROS generators such as NAD(P)H oxidases 1, 2, and 4 (NOX 1, 2, 4) is also low. On differentiation, mitochondrial respiratory activity tends to increase, leading to increase in ROS generation and changes in expression of nitric oxide synthases 1–3 (NOS-1, 2, and 3). Antioxidants catalase (Cat), Mn-superoxide dismutase (SOD2), glutathione peroxidases 2, 3, and 4 (Gpx2–4) expression decreases while expression of Cu-Zn superoxide dismutase (SOD1), Gpx1, and peroxiredoxins 1 and 2 (Prx1, 2) increase. Compared with the differentiated normal cells with higher ROS ($ROS^{mid}$) than stem cells, cancer cells may exhibit even higher ROS ($ROS^{high}$) levels because of active metabolism, oncogene stimulation, and other genetic alterations. It is unclear now whether the redox status in cancer stem cells may be similar to that of normal stem cells ($ROS^{low}$) to maintain stemlike features. *Mice, ΩHuman and mice, ~increases followed by decreasing as differentiation progresses.

important to consider whether any of the major signaling pathways important in regulating normal stem cells are redox sensitive and may be applicable to cancer stem cells. The role ROS have in regulating HSC functions and the pathways susceptible to ROS were reviewed recently by Naka *et al.* (72).

The Wnt/$\beta$-catenin signaling pathway in stem cell biology has been demonstrated to be important in regulating stem cell proliferation and renewal [reviewed in (87)]. On binding of Wnt proteins to the Frizzled receptors, disheveled (Dvl) is activated, to release $\beta$-catenin *via* inhibition of glycogen synthase kinase-3$\beta$. Free $\beta$-catenin moves to the nucleus to bind to the Lef/Tcf family of transcription factors to activate transcription of its target genes (87). Generation of a conditional knockout for $\beta$-catenin in a mouse hematopoietic system revealed a reduced capacity for long-term HSC self-renewal (120). Their study also revealed, through induction of CML *via* a BCR-ABL model of leukemogenesis, the role $\beta$-catenin may have in the progression of myeloid leukemia cells *in vivo* (120). The Wnt/ $\beta$-catenin pathway, important for transcription of various genes, has been found to be sensitive to ROS. Hydrogen peroxide was found negatively to regulate Wnt signaling through downregulation of $\beta$-catenin, whereas supplementation with Wnt3a overcame this effect (94).

### *Redox in self-renewal and differentiation*

Studies have found ROS to influence the self-renewal capability of stem cells. For example, Ito *et al*. (45) found increased ROS generation in $ATM^{-/-}$ hematopoietic stem cells (HSCs) to be responsible for decreased self-renewal capability through activation of the $p16^{INK4a}$ pathway. Incubation with the antioxidant *N*-acetyl-cysteine (NAC) reduced ROS levels and improved hematopoietic reconstitution. The study concluded that activation of the $p16^{INK4a}$-Rb pathway inhibited phosphorylation (inactivation) of the retinoblastoma protein (Rb), which affected $ATM^{-/-}$ HSC self-renewal (45). The same group also found that activation of the p38 MAPK pathway through ROS decreased the self-renewal capacity of HSCs (46).

Further studies of the stem-cell niche support the importance of a proper redox state in maintenance of stem cells. Stem cells reside in a relatively hypoxic (low oxygen) environment and appear to require it in part to maintain its population and self-renewal potential (25, 47, 95). Isolation of HSCs based on ROS levels (low, moderate, and high) showed that $ROS^{low}$ cells possess greater secondary and tertiary reconstitution capacity in lethally irradiated mice compared with $ROS^{high}$ cells in a competitive reconstitution assay (47). These results underscore the significance of the environment, particularly hypoxia, in maintaining stem cell function. In addition, hypoxia regulates transcription factors such as HIFs and other proteins that influence stem cell behavior [reviewed in (95)].

Maintenance of pluripotency by redox-sensitive signaling molecules has also been implicated (95). One example of the role ROS play in differentiation is in cardiomyogenesis. Embryonic stem cell–derived embryoid bodies treated with antioxidants NAC, catalase, and the NOX inhibitor diphenyleneiodonium (DPI) showed reduced percentage of beating embryoid bodies (EBs)/cardiomyocytes. In contrast, a dose-dependent addition of up to 100 n*M* $H_2O_2$ appeared to stimulate the percentage of beating EBs (10). Further study found the cytokine cardiotrophin-1, which regulates a variety of functions in cardiomyocytes, particularly differentiation, is regulated by ROS and the transcription factor, HIF-1 (hypoxia-inducible factor) (4).

### *Role of transcription factors in resistance to oxidative stress*

As described earlier, the environment is pertinent to stem cell regulation and function. In addition to antioxidants, various transcription factors are also influenced by ROS and function in stem cell maintenance.

NF-E2–related factor-2 (Nrf2) is a member of the CNC-bZIP family of proteins, which binds to the antioxidant response element (ARE) to activate the transcription of a variety of antioxidant proteins. Nrf2 is found associated with Keap1 in the cytoplasm, where it is targeted for degradation. Translocation of Nrf2 to the nucleus occurs on dissociation with Keap1 under oxidative stress or through the use of electrophilic agents. For example, on treatment of $Nrf2^{+/+}$ cardiac fibroblasts with 3*H*-1,2-dithiole-3-thione (D3T), a variety of antioxidants such as superoxide dismutase (SOD), catalase, glutathione (GSH), glutathione reductase (GR), glutathione peroxidase (GPx), and the phase 2 detoxifying enzymes NAD(P)H:quinone oxidoreductase-1 (NQO1) were induced (122). However, such regulatory activity was abolished in $Nrf2^{-/-}$ cells, leaving them more susceptible to oxidative stress. Given the significant role Nrf2 plays in protecting cells, studies on knockout models provide insight into its role during embryonic development. Mice with $Nrf1^{-/-}$ genes were found to be anemic and embryonic lethal (15, 18), whereas $Nrf2^{-/-}$ were found to be viable (16). In addition, Leung *et al.* (59) found compound Nrf1/Nrf2 knockouts to be embryonic lethal, with increased ROS levels compared with single Nrf1 or Nrf2 knockouts. Such studies highlight the distinct function of Nrf1 and Nrf2 genes in embryonic development, with certain overlapping roles in ROS regulation.

As ROS influences stem cell self-renewal and differentiation, transcription factors such as those of the FoxO (Forkhead box O) subfamily protect stem cells from oxidative stress (103). A conditional knockout of three FoxO alleles, FoxO1, 3, and 4 by a Mx-Cre transgene system in HSCs, showed increased intracellular ROS compared with $Mx\text{-}Cre^{-}$ mice. Interestingly, the study found the increase in ROS to be in the HSC subset, with no difference in intracellular ROS levels between WT and FoxO-deficient myeloid progenitors (102). Further gene-expression studies revealed changes in expression of antioxidant genes such as SOD1 and SOD3 compared with WT cells. Daily treatment with the antioxidant, *N*-acetylcysteine (NAC), resulted in lower ROS levels and reduced phenotypic defects of the HSCs. Restoration of the HSCs by NAC supports that the deficiencies were due, in part, to increased ROS levels in the Fox-O–deficient cells (102).

Transcription factors, which have a direct role in stem cell development, such as Oct-4, have also been found to be redox regulated. Oct-4 is found to be important for maintaining stem cell totipotency and found to be downregulated on differentiation (81, 82). Guo *et al.* (34) found Oct-4 to associate directly with the antioxidant Trx, which restored DNA binding of Oct-4 after oxidation with diamide. In addition, Trx overexpression increased Oct-4 transcriptional activity, whereas dominant negative Trx showed reduced activity. The

authors concluded that Trx is important in protecting Oct-4 from oxidizing agents.

From the examples described, one can surmise the significance of transcription factors in regulating stem cell function not only through activation of antioxidants on oxidative stress, but also through direct association with antioxidant proteins. Knockout models further implicate the significance of these transcription factors by being embryonic lethal or more susceptible to oxidative stress.

## Therapeutic Approaches

### *Redox targeted cancer therapy*

Increased generation of ROS, defects in antioxidant mechanisms for ROS elimination, or a combination of both may result in a state of oxidative stress in cancer cells. The highly reactive properties of ROS have profound effects in the cells, specifically, when in greater concentrations, it may cause DNA damage, protein oxidations, and trigger apoptosis or necrosis (105). Currently, two main redox-targeting strategies are known for cancer: antioxidant therapy and oxidant therapy. Clinical studies of both strategies show some levels of therapeutic potential and limitations (11, 77). The different modes of cancer redox imbalance, as described in Fig. 2, might result in different therapeutic outcomes. Different cellular oxidation capacities and redox adaptations in the cancer cells may be the determining factors for redox-targeted therapeutic sensitivity (Fig. 4). Therefore, an effective redox-targeting therapeutic strategy will be able to (a) promote redox stress to the point of cell death and (b) suppress the cancer cell ability to adapt to redox stress (80). Targeting the redox imbalance found in cancer cells has been shown to be feasible. Investigations on the therapeutic potential of the natural product, $\beta$-phenylethyl isothiocyanate (PEITC), exemplified an effective method of killing cancer cells *via* a redox-altering mechanism (104, 106, 119). Alternatively, inhibition of antioxidants such as SODI, or the transcription factor Nrf2, increased the sensitivity of cancer cells to chemotherapeutic agents (41, 112). Resistance to ROS-generating agents such as doxorubicin and cisplatin was found to be related to elevated levels of the antioxidant, GSH (9). Thus, use of the GSH-depleting agent, buthionine sulfoximine (BSO), has been shown to enhance sensitivity of cancer cells to chemotherapeutic agents [reviewed in (11)]. Constitutively expressed and

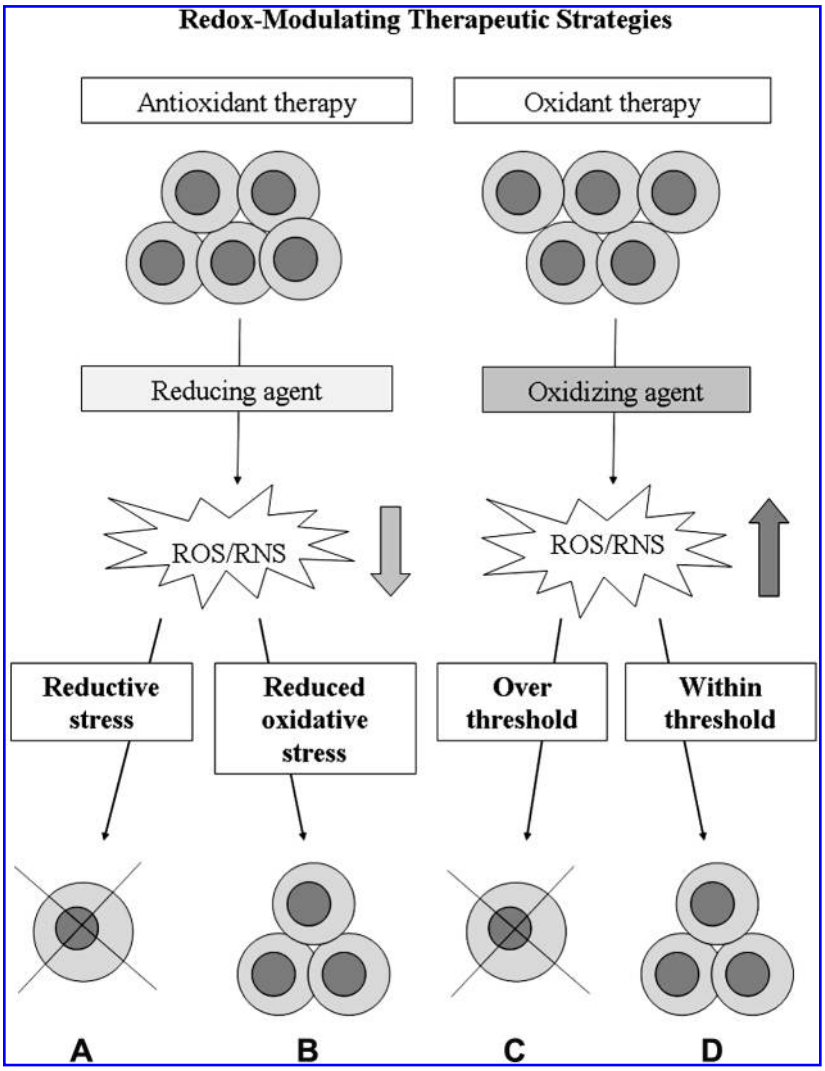


**FIG. 4. Redox-modulating therapeutic strategies for cancer treatment**. Treatment of cancer cells through the use of reducing agents will lead to reduction of ROS/RNS, leading to either (**A**) cell death *via* reductive stress or (**B**) release cancer cells from oxidative stress, leading to increased cell survival and therapeutic failure. Conversely, drugs that increase ROS generation may induce severe oxidative damage that surpasses the threshold for cell survival, causing massive cancer cell death (**C**). Increased redox adaptation in certain cancer/cancer stem cells will allow cell survival despite oxidant generation because of a higher threshold or elevated capacity to withstand ROS/RNS (**D**). This may be an important mechanism contributing to therapy failure.

Table 1. Functions of Constitutive Antioxidants and Inducible Antioxidants

| *Endogenous antioxidants* | *Main antioxidant functions* | *References* |
|---|---|---|
| Constitutive antioxidants[a] | | |
| Nonthiol antioxidants | | |
| SOD1, 2 | $O_2^{-}\cdot$ scavenger | 36 |
| Cat | $H_2O_2$ scavenger | 50 |
| Thiol-dependent antioxidant | | |
| GPXs | GSH metabolism<br>$H_2O_2$ and lipid peroxides scavenger | 28 |
| Inducible antioxidants | | |
| Nonthiol antioxidants | | |
| Cat[c] | $H_2O_2$ scavenger | 12 |
| HO-1[b] | Heme degradation yields reductants biliverdin and bilirubin | 48 |
| SOD2[c] | $O_2^{-}\cdot$ scavenger | 36 |
| Thiol-dependent antioxidants | | |
| GCL[b] | GSH synthesis, increase GSH content | 33 |
| Grxs[b] | Electron donor to sulfur molecule reductions<br>Protein glutathionylation and disulfide formation | 62 |
| Prxs[b] | $H_2O_2$ scavenger | 51 |
| Srx[b] | Electron donor to sulfur molecule reductions | 27 |
| Trxs[b] | $H_2O_2$ scavenger<br>Electron donor to thiol-dependent antioxidant | 84 |

[a]Stably expressed antioxidants under normal physiologic conditions.
[b]Transiently induced by chemicals, ROS/RNS, hypoxia, radiation, oxidative-damaged molecules.
[c]Upregulated expression or increased activity under continuous oxidative stress.

inducible antioxidants, as listed in Table 1, are possible targets for redox therapy.

### Targeting Cancer Stem Cells

The possible existence of cancer stem cells (CSCs), a subpopulation of cancer cells that possess stem cell–like features, is perhaps one of the primary reasons for drug resistance and tumor recurrence. Developing strategies for the elimination of CSCs have led to a surge in understanding of their biology relative to that of normal stem cells and the remaining tumor bulk. Studies of cancer cells indicate that they possess a greater capacity to resist oxidative stress, such as resistance to certain chemotherapeutic agents (29). A study by Saretzki *et al.*

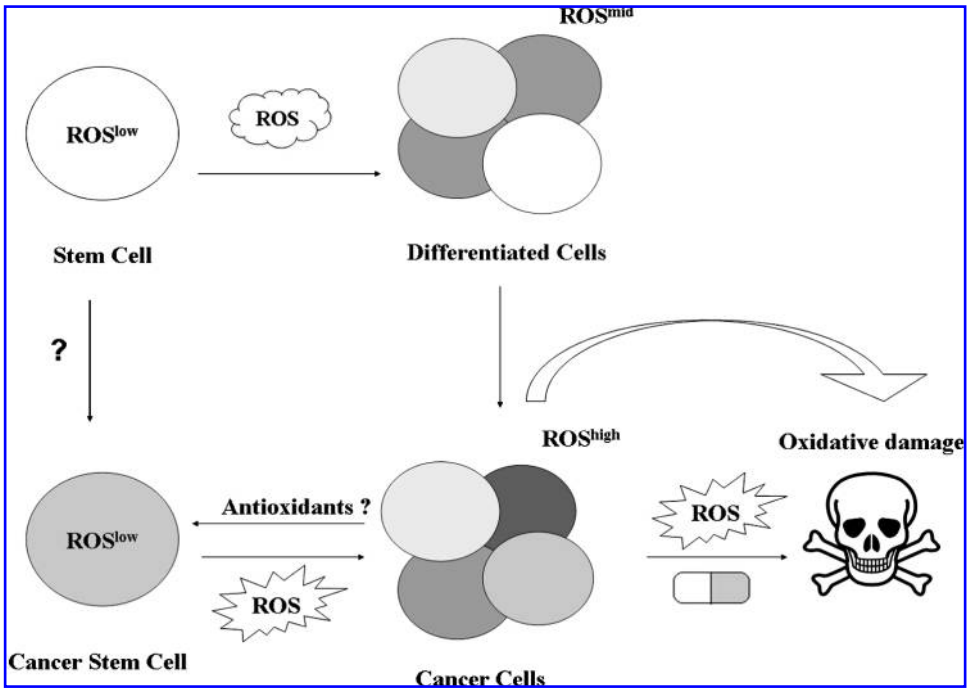


**FIG. 5. A redox-targeted therapeutic approach to killing cancer stem cells.** Stimulation of stem cells to differentiate by ROS has been shown to be possible. It is still unclear whether a cancer stem cell evolves from a normal stem cell and whether its redox status will be similar to normal stem cells or to the "regular" cancer cells in the tumor bulk. It is unknown whether ROS generation or antioxidant inhibition may stimulate cancer stem cells to differentiate to "regular" cancer cells, which can then be targeted by other chemotherapeutic agents (*i.e.,* ROS generators, antioxidant inhibitors) to cause cell death. Cloud ROS, physiologic ROS; explosive ROS, aberrant ROS (*i.e.*, drug induced).

(91) found that DNA damage induced by ionizing radiation to mES cells had greater DNA-repair capacity than the differentiated counterpart (91). Similarly, $CD133^+$ glioma CSCs were found to survive better against ionizing radiation compared with $CD133^-$ cells because of a more efficient DNA-repair response (89). Additionally, two studies recently showed that $CD133^+$ CSCs conferred chemoresistance to doxorubicin and cisplatin (known ROS generators) in hepatocellular carcinoma and ovarian cancer cells, respectively (5, 64).

Identifying similar and differing characteristics between stem cells and cancer stem cells may allow the development of a strategy in specifically eliminating CSCs. Current therapeutic approaches proposed for the elimination of CSCs involve targeting signaling pathways required for the maintenance of self-renewal and differentiation capacities. Given the sensitivity of stem cells and its modulators to redox alterations, perhaps CSCs may also be sensitive to redox alterations. Compounds that induce differentiation have been studied both in stem cells and in leukemia. Retinoic acid (RA) has been used as a form of differentiation–induction therapy in acute myeloid leukemia (58). The use of retinoic acid in murine embryonic stem cells was found to inhibit the leukemia-inhibitory factor (LIF) signaling pathway, including STAT3 tyrosine phosphorylation, resulting in induction of differentiation (101). Another study found that the use of RA on embryoid bodies induced apoptosis *via* ROS generation, but induced differentiation in undifferentiated cells (14). Interestingly, a recent study revealed that RA maintained self-renewal of mESCs *via* a feedback mechanism of RA-induced LIF and Wnt signaling (111).

## Concluding Remarks

The potential use of ROS generation or inhibition of antioxidants as a therapeutic strategy in elimination of CSCs can come about only through investigating the redox status specifically in these cells (Fig. 5). Although many cancer cells show an increase in ROS, it is unknown whether CSCs would also retain this phenotype or maintain a lower ROS state. Perhaps CSCs would maintain a higher reductive potential to maintain "stemness," just as ES cells require a low ROS status for maintaining self-renewal and pluripotency. In this case, induction of ROS stress in the stemlike cancer cells by pharmacologic agents might cause the CSCs to lose their stemness and thus improve long-term therapeutic outcome.

## Abbreviations

ARE, Antioxidant response element; ATM, ataxia-telangiectasia mutated; BSO, buthionine sulfoximine; Cat, catalase; CSCs, cancer stem cells; CLL, chronic lymphocytic leukemia; DCF, dichlorofluorescein; DCF-DA, dichlorofluorescein-diacetate; 2,3-DHBA, 2,3-dihydroxy benzoic acid; DPI, diphenyleneiodonium; DUOX, dual oxidase; EBs, embryoid bodies; EM, electron micrograph; ER, endoplasmic reticulum; ERO1-L, ER oxidoreductin 1–like protein; FoxO, forkhead box O; G6PDH, glucose-6-phosphate dehydrogenase; GCL, γ-glutamyl-cysteine synthetase; Grx, glutaredoxin; γ-GT, γ-glutamyltranspeptidase; GSH, glutathione; GPX, glutathione peroxidase; GR, glutathione reductase; GS, glutathione synthase; GSNO, *S*-nitrosoglutathione; GST, glutathione-*S*-transferases; hESCs, human embryonic stem cells; HSC, hematopoietic stem cells; HO, heme oxygenase; •HO, hydroxyl radical; HPSCs, hematopoietic stem and progenitor cells; LIF, leukemia inhibitory factor; mESCs, murine embryonic stem cells; MSCs, mesenchymal stem cells; mGPDH, mitochondrial glycerophosphate dehydrogenase; NAC, *N*-acetyl-cysteine; NO• , nitric oxide; NOS, nitric oxide synthase; NOX, NAD(P)H oxidase; Nrf2, NF-E2–related factor-2; PDI, protein disulfide isomerase; -ONOO, peroxynitrite; Prx, peroxiredoxins; PHGPX, phospholipid hydroperoxide; PTEN, phosphatase and tensin homologue; RA, retinoic acid; RNS, reactive nitrogenous species; ROS, reactive oxygen species; $O_2^-$•, superoxide; SOD, superoxide dismutase; Srx, sulfiredoxin; Trx, thioredoxin; TrxR, thioredoxin reductase.

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