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Review

New insights into redox regulation of stem cell self-renewal and differentiation☆

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ABSTRACT

Background: Reactive oxygen species (ROS), the natural byproducts of aerobic metabolism, are precisely orchestrated to evoke diverse signaling pathways. To date, studies have focused mainly on the detrimental effects of ROS in stem cells. Recently, accumulating evidence has suggested that ROS also function as second messengers that modulate stem cell self-renewal and differentiation by regulating intricate signaling networks. Although many efforts have been made to clarify the general effects of ROS on signal transduction in stem cells, less is known about the initial and direct executors of ROS signaling, which are known as 'redox sensors'.
Scope of review: Modifications of cysteine residues in redox sensors are of significant importance in the modulation of protein function in response to different redox conditions. Intriguingly, most key molecules in ROS signaling and cell cycle regulation (including transcriptional factors and kinases) that are crucial in the regulation of stem cell self-renewal and differentiation have the potential to be redox sensors.
Major conclusions: We highlight herein the importance of redox regulation of these key regulators in stem cell self-renewal and differentiation.
General significance: Understanding the mechanisms of redox regulation in stem cell self-renewal and differentiation will open exciting new perspectives for stem cell biology. This article is part of a Special Issue entitled Redox regulation of differentiation and de-differentiation.

1. Introduction

Stem cells are defined as cells that possess the capacity for self-renewal and differentiation to maintain 'stemness'. Self-renewal refers to the ability to divide into at least one daughter cell that is identical to the parent cells, while differentiation describes the ability to differentiate into a variety of cell lineages and tissues [1]. Since the balance between stem cell self-renewal and differentiation controls stem cell fate, clarification of the involved molecular mechanisms would assist the application of stem cells in regenerative medicine [2].

Reactive oxygen species (ROS), the highly chemically reactive byproducts of aerobic metabolism, are important mediators in stem cell biology [3,4]. Elevated intracellular ROS was initially recognized to be toxic and associated with cell death. As early as 2000, Smith's group reported that the intracellular redox status appears to be a necessary and sufficient modulator to maintain the balance between self-renewal and differentiation in dividing glial precursor cells [5]. During the last decade, mounting experimental evidence suggests that stem cells undergoing self-renewal reside in niches with low levels of ROS, whereas in differentiated stem cells, ROS is accumulated [6,7]. For instance, iPSCs with decreased levels of free radical damage and antioxidant enzymes were arrested in the self-renewal state: the differentiated iPSCs contain more oxidative proteins than those in the undifferentiated state [8]. These studies imply that redox signaling plays a crucial role in modulating the fate of stem cells. A number of recent observations have confirmed that low levels of ROS maintain 'stemness', whereas higher levels of ROS promote differentiation in different types of stem cells [9–11]. Although significant progress has been made, exactly how ROS monitor the balance of stem cell self-renewal and differentiation is still not adequately understood. It is becoming clear that redox modifications of cysteine residues are pivotal mechanisms for the functional regulation of almost all major protein classes, and correlate with many disease states [12]. ROS signaling and cell cycle progression modulated by key regulators are crucial for regulating stem cell self-renewal and differentiation [7,13,14], and most of these key regulators (including transcriptional factors and kinases) are susceptible to redox changes

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and are recognized as redox sensors (Table 1). Understanding the roles that redox sensors play will confer important biological insights to assist in deciphering the molecular mechanisms regulating stem cell self-renewal and differentiation.

2. Redox modifications of protein thiols

While ample evidence has been generated to support the pivotal role of ROS in the regulation of cellular signaling pathways, exactly how ROS initiated the signaling remained unclear until scientists began to focus on oxidative modifications of cysteine in redox sensors [12]. Such oxidative modifications commonly occur on the active thiol groups (R–SH) of cysteine residues. In response to oxidation, cysteine thiol can reversibly form sulfenic acid (R–SOH), intramolecular/intermolecular disulfide bonds (R–S–S–R/R–S–S–R′) or mixed disulfides with glutathione (R–S–S–G, known as S-glutathionylation), which can be reduced back to thiol by reductants such as thioredoxin, glutaredoxin, peroredoxin (*in vivo* reductants) or dithiothreitol (*in vitro* reductant) [15]. The presence of reactive nitrogen species (RNS) leads to the formation of S-nitrosothiol (R-SNO) or S-nitrothiol ($R\text{-}SNO_2$). Sulfenic acid can be further irreversibly oxidized to sulfinic ($R\text{-}SO_2H$) or sulfonic ($R\text{-}SO_3H$) acid [16] (Fig. 1). These thiol-based redox switches subtly modulate the function of redox sensors and directly affect biological processes, starting from gene transcription, translation and protein folding, to metabolism, signal transduction, and ultimately apoptosis [17,18]. Hence, it has become increasingly important to assess the redox status of protein thiols. A large number of proteins have been identified as redox sensors over the past few years, some of which are implicated in the regulation of stem cell self-renewal and differentiation, including transcriptional factors and kinases that are involved in ROS signaling and cell cycle regulation.

3. The role of transcription factors in redox regulation of stem cell self-renewal and differentiation

Stem cells undergoing self-renewal process have been shown to reside in niches with low levels of ROS [19]. Endogenous antioxidant enzymes are critical defense mechanisms against oxidative stress to maintain intracellular ROS homeostasis in stem cells [20]. The antioxidant enzyme systems in stem cells are monitored by key transcription factors such as the forkhead box protein O (FoxO) family and nuclear factor erythroid-2-related factor 2 (Nrf2) [21] (Fig. 2).

3.1. FoxOs

Increasing evidence has shown that FoxO family members, including FoxO1, FoxO3 and FoxO4, are important mediators in regulating the self-renewal of neural stem cells (NSCs) [22,23], embryonic stem cells (ESCs) [24] and hematopoietic stem cells (HSCs) [25]. In particular, FoxO3 maintains HSC self-renewal by enhancing the expression of superoxide dismutase 1/2/3 (SOD1/2/3) and catalase (both of which are its target genes) resulting in decreased intracellular ROS levels [26,27]. FoxO family members contain two highly conserved cysteines, one of which is Cys477 in FoxO4 that can form an intermolecular disulfide bridge with p300/CBP acetyltransferase, which alleviates FoxO4-induced cell cycle arrest and simultaneously enhances FoxO4-induced apoptosis [28]. In addition, a recent report suggests that H_2O_2 (exogenous ROS) or glucose deprivation (endogenous ROS) can also induce disulfide formation in Cys239 of FoxO4 with transportin-1, which is required for nuclear localization and transcriptional activity [29]. Since FoxOs can regulate stem cell self-renewal by diminishing intracellular ROS level, the potential remains for further investigation into whether FoxOs can function as redox sensors to regulate stem cell self-renewal and differentiation.

3.2. Nrf2

Similarly, Nrf2 can drive antioxidant response by activating expression of antioxidant enzyme genes [30]. Kelch-like ECH-associated protein 1 (Keap1) is an actin-binding cytoplasmic protein that binds to Nrf2 in cytoplasm and facilitates the proteasomal degradation of Nrf2 [31]. Recent findings suggest that Nrf2 stabilization profoundly protects mesenchymal stem cells (MSCs) and intestinal stem cells (ISCs) against oxidative stress [32,33]. By contrast, in HSCs, Nrf2-regulated stem cell survival is found to be independent of intracellular ROS levels [34]. These important results suggest that Nrf2 is implicated in regulating

Table 1
Key regulators that can be oxidized in response to oxidative stress in the regulation of stem cell self-renewal and differentiation.

Regulators	Oxidized cysteine residues	Modifications	Alteration of protein function	References
FoxO4	Cys477	Intermolecular disulfide	Switching from cell cycle arrest to apoptosis induction	[24]
	Cys239	Intermolecular disulfide	Activation	[25]
Nrf2	Cys183	Unknown	Activation	[31–33]
	Cys119, Csy235, Cys506	Unknown	Inactivation	[31–33]
Keap1	Cys151, Cys273, Cys288	Intramolecular or intermolecular disulfide	Inactivation	[34–36]
p53	Cys275, Cys277,	Intramolecular disulfide	Inactivation	[51,52]
	Cys124, Cys141, Cys182	S-glutathionylation	Inactivation	[53,54]
HIF-1α	Cys800	S-nitrosylation	Activation	[41]
	Cys800	S-nitrosylation	Inactivation	[43]
	Cys533	S-nitrosylation	Activation	[42]
STAT3	Cys418, Cys426, Cys468	S-glutathionylation	Inactivation	[55,56]
NF-κB subunit p50	Cys62	S-glutathionylation	Inactivation	[59]
IKKβ	Cys179	S-glutathionylation	Inactivation	[60]
Oct4	Unknown	Intermolecular disulfide	Inactivation	[64]
Ref-1	Cys65, Cys93	Intramolecular disulfide	Inactivation	[31,66–69]
PI3K	Unknown	Sulfenic acid	Inactivation	[77]
Akt1	Cys310	Sulfonic acid	Inactivation	[78]
Akt2	Cys297, Cys311	Intramolecular disulfide	Inactivation	[79,80]
PTEN	Cys71, Cy124	Intramolecular disulfide	Inactivation	[81]
	Unknown	S-nitrosylation	Inactivation	[82]
JNK1	Cys116	S-nitrosylation	Inactivation	[86]
p38	Cys39, Cys119, Cys162, Cys211	Unknown	Inactivation	[87]
ATM	Cys2991	Intermolecular disulfide	Activation	[95,96]
Cdc25C	Cys330, Cys377	Intramolecular disulfide	Inactivation	[100]

Abbreviations: Cys, cysteines; FoxO4, forkhead box protein O4; Nrf2, nuclear factor erythroid-2-related factor 2; Keap1, kelch-like ECH-associated protein 1; HIF-1α, hypoxia-inducible factor 1α; STAT3, signal transducer and activator transcription factor 3; NF-κB, nuclear factor kappa B; IKKβ, IκB kinase; Ref-1, redox factor-1; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog; JNK, c-Jun N-terminal kinase; ATM, ataxia telangiectasia mutated.

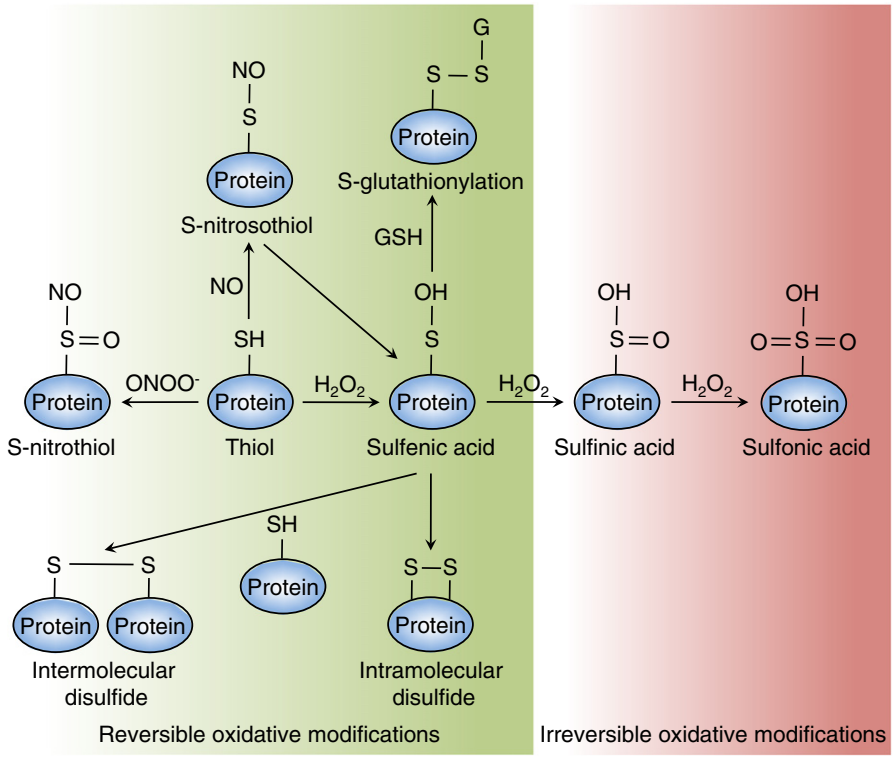

Fig. 1. Redox modifications of protein thiols. Cysteine thiol can be reversibly oxidized to form unstable sulfenic acid (R-SOH) by H_2O_2. Alternatively, thiol can also be oxidized by RNS such as NO or peroxynitrite ($ONOO^-$) to yield S-nitrosothiol (R-SNO) or S-nitrothiol ($R\text{-}SNO_2$), respectively. Sulfenic acid can then be stabilized by reacting with nearby thiols to form intracellular/intercellular disulfide bond (R–S–S–R/R–S–S–R′) or with glutathione leading to formation of mixed disulfide (R–S–S–G). These oxidative modifications are reversible and can be reduced back to thiol by reductants. Higher level of H_2O_2 can promote over-oxidation forming sulfinic ($R\text{-}SO_2H$) or sulfonic ($R\text{-}SO_3H$) acid, both of which are irreversible.

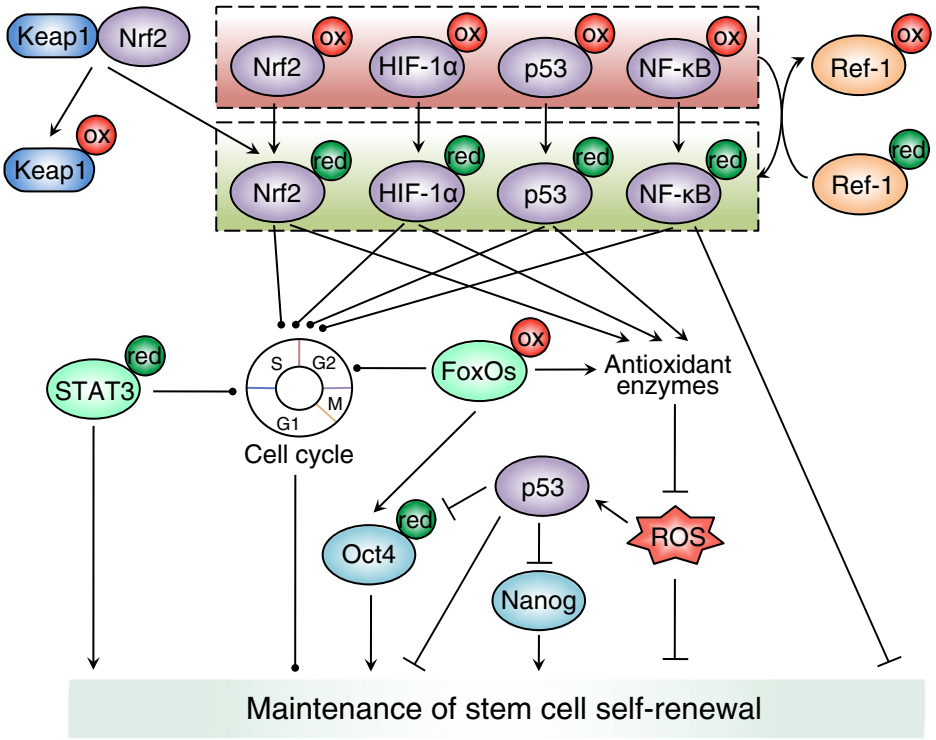

Fig. 2. Transcription factors in redox regulation of stem cell self-renewal. The activity of key transcription factors including Nrf2, HIF-1α, p53 and NF-κB can be enhanced by switching the oxidized to the reduced state in the presence of reduced Ref-1. These reduced transcription factors, as well as reduced STAT3 and oxidized FoxOs, are all involved in ROS signaling and cell cycle regulation, both play critical role in the regulation of stem cell self-renewal and differentiation. p53 can also act in the downstream signaling molecules of ROS to inhibit the expression of Oct4 and Nanog. In addition, FoxOs regulate the stem cell self-renewal, at least in part, due to the enhanced expression of Oct4. 'Ox' denotes the oxidized state, while 'red' denotes the reduced state. Abbreviations: Nrf2, nuclear factor erythroid-2-related factor 2; HIF-1α, hypoxia-inducible factor 1α; NF-κB, nuclear factor kappa B; Ref-1, redox factor-1; STAT3, signal transducer and activator transcription factor 3; FoxO4, forkhead box protein O4; ROS, reactive oxygen species; Keap1, kelch-like ECH-associated protein 1.

stem cell self-renewal both in a ROS-dependent and ROS-independent manner. Intriguingly, Nrf2 is also recognized as a redox sensor by Cys506 and Cys183 oxidation, which inhibits its binding affinity with antioxidant responsive element (ARE) enhancer and induces its nuclear localization, respectively [35,36]. In addition, Cys119 and Csy235 in Nrf2 are also susceptible to oxidation, leading to Keap1-dependent ubiquitination-proteasomal degradation [1]. As the common inhibitory protein for Nrf2, Keap1 has been well documented to be a redox sensor by forming intramolecular or intermolecular disulfide bridges in Cys151, Cys273 and Cys288. These oxidative modifications contribute to dissociation of Keap-1 from Nrf2 and subsequent accumulation of Nrf2 [37–39].

3.3. HIF-1α

Stem cells have been shown to live in hypoxia niches to maintain undifferentiated states [40]. Hypoxia-inducible factor 1α (HIF-1α), a well-known transcription factor in response to hypoxia, has been highlighted as a key regulator in stem cell self-renewal and differentiation in recent studies. In a low-oxygen microenvironment, HIF-1α endows HSCs with the potential for metabolic adaption by its transcriptional activation [41], or maintenance of cell cycle quiescence by regulating p16(Ink4a) and p19(Arf) [42]. HIF-1α is also reported to enhance self-renewal activity and inhibit differentiation in cancer stem cells (CSCs) upon exposure to hypoxic stress [43]. Evidence indicates that HIF-1α is also a target for S-nitrosylation in response to NO under normal oxygen tensions. S-nitrosylated Cys800 and Cys533 have been reported to contribute to HIF-1α stabilization and activation [44,45]. However in another report using purified proteins and peptides, Cys800 S-nitrosylation abrogates the interaction of p300 co-activator with HIF-1α, resulting in almost complete reversal of HIF-1α activation [46]. These apparently contradictory results may be due to the differential oxygen content of the environment.

3.4. p53

The balance of stem cell self-renewal and differentiation requires coordination of the cell cycle control [13]. In particular, ESCs undergoing self-renewal have a shortened cell cycle allowing proliferation at high rates owing to truncated G1 phase, while in contrast to ESCs, adult stem cells repeat periods of quiescence and cell cycle re-entry to maintain stem cell function [1,13]. p53 has been reported to promote differentiation of ESCs by cell cycle regulation [47]. Interestingly, the regulation of differentiation by p53 is dependent upon p53-activating genes in mouse ESCs, but independent of the activation of its target genes in human ESCs [48,49]. Moreover, in Sirtuin $1^{-/-}$ mouse ESCs, endogenous ROS induced by 2-mercaptoethanol withdrawal can induce the translocation of p53 into the nucleus to inhibit Nanog expression, while in wild type ESCs, p53 is translocated to the mitochondria stimulating apoptosis. These results suggest that endogenous ROS-controlled p53 subcellular localization is important for maintenance of mouse ESCs [50]. In addition to ESCs, p53 is also implicated in regulating self-renewal and differentiation of other types of stem cells, including NSCs [51] and HSCs [52,53]. p53 shows remarkable redox-sensitivity and is a well-defined redox sensor. Oxidized Cys275 and Cys277 can form a reversible intermolecular disulfide bond under mild oxidizing conditions that profoundly decreases the DNA binding capacity of p53 [54,55]. Other cysteines, including Cys124, Cys141, and Cys182 which are all present in the proximal DNA-binding domain, have been identified as the sites of S-glutathionylation by mass spectrometry, with Cys141 being the most reactive cysteine on the p53 surface [56,57]. S-glutathionylation of these cysteines also impedes the binding of p53 to DNA as well as p53 dimerization [56,57]. These reports suggested that redox modifications of p53 are of significant importance in modulating its function.

3.5. STAT3 and NF-κB

In a similar manner to p53, the transcriptional activity of signal transducer and activator transcription factor 3 (STAT3) and nuclear factor kappa B (NF-κB), both of which are key transcription factors for cell cycle regulation, are also inhibited by S-glutathionylation. S-glutathionylation of STAT3 at Cys418, Cys426 and Cys468 profoundly decreases its DNA binding activity and impairs its function on cell proliferation and survival [58,59]. Moreover, leukemia inhibitory factor (LIF)-driven activation of STAT3 has been demonstrated to prevent differentiation and maintain pluripotency and self-renewal in mouse ESCs [60,61]. These important findings imply that redox modification of STAT3 might be involved in the regulation of stem cell self-renewal and differentiation (Fig. 2). The DNA binding activity of NF-κB can also be disrupted by S-glutathionylation of p50 at Cys62, a functional subunit of NF-κB [62]. More recently, IκB kinase (IKKβ), which phosphorylates the inhibitor IκB to release NF-κB for nuclear translocation and subsequent gene transcription, has been found to be a central target for oxidative inactivation by means of S-glutathionylation of Cys179 [63]. NF-κB has also been suggested as a critical regulator in stem cell differentiation. It is found that the activity of NF-κB is relatively low in undifferentiated human ESCs, but increases during ESCs differentiation [64]. Another report on human MSCs shows that NF-κB activation promotes osteogenic differentiation [65].

3.6. Oct4

Oct4, a homeodomain transcription factor, is one of the key regulators required in the maintenance of pluripotency and self-renewal of ESCs, and is frequently used as a marker for undifferentiated cells [66]. It has been reported that self-renewal of ESCs can be positively controlled by FoxO1-induced Oct4 gene expression [24], or negatively controlled by p53-repressed Oct4 expression [47]. Intriguingly, DNA binding activity of Oct4 is sensitive to abrogation by oxidizing cysteines in the POU domain, which can be reversed by thioredoxin, suggesting that Oct4 is also a redox sensor [67].

3.7. Ref-1

The mammalian apurinic/apyrimidinic (AP)-endonuclease, redox factor-1 (Ref-1), is a dual function protein that plays essential roles both in DNA repair and regulation of the redox state of transcription factors [68]. Ref-1 acts as a redox sensor that monitors intracellular ROS levels by modulating the activity of several transcription factors, including Nrf2, HIF-1α, p53 and NF-κB, all of which can activate antioxidant enzyme gene expression [35,69–71]. In this regard, reduced Ref-1 can directly switch oxidized Nrf2, HIF-1α, p53 or NF-κB to the reduced state to enhance their transcriptional activities. The redox switches of these transcription factors are accomplished by shifting Cys65 and Cys93 of Ref-1 from the reduced to the oxidized state [72] (Fig. 2). Hence, it can be speculated that Ref-1-regulated ROS is implicated in the balance of stem cell self-renewal and differentiation. Indeed, Ref-1 is critical in the regulation of hematopoietic differentiation of ESCs through its redox functional domain, but not by DNA repair [73]. In cardiac stem cells, Ref-1 inhibition followed by H_2O_2 treatment enhanced intracellular ROS levels and induced oxidative injury-mediated cardiac cell death and differentiation, while treatment with the ROS scavenger N-acetyl-L-cysteine (NAC) could attenuate this effect [10]. It has also been reported that overexpression of Ref-1 can rescue human bone marrow derived mesenchymal stem cells (hBMSCs) from senescence by suppressing the production of intracellular ROS [74]. However, whether redox switching of transcription factors by Ref-1 is involved in Ref-1-regulated ROS in stem cell self-renewal and differentiation remains unclear.

3.8. The modulation of redox-sensitive transcription factors by resveratrol in stem cell differentiation

Resveratrol is a natural antioxidant compound that has the potential to counteract free radicals to protect cells from oxidative damage [75]. Recent studies have demonstrated that resveratrol could regulate redox-sensitive transcription factors (including p53, FoxO3A, Nrf2 and Ref-1) that are crucial for the survival and differentiation of stem cells. For instance, resveratrol activated the p53/p21 pathway and promoted proteasomal degradation of Nanog, resulting in increased differentiation capacity in glioma stem cells (GSCs) [76]. Resveratrol can also activate the SIRT1/FoxO3A axis, whereby expression of the osteo-lineage gene RUNX2 was upregulated to promote the differentiation and osteogenesis of human mesenchymal stem cells [77]. However, there is little discussion of the antioxidant effect of resveratrol in stem cell differentiation in these two studies. In an earlier study, Gurusamy et al. demonstrated that resveratrol treatment could induce the expression of Nrf2 and Ref-1 in rats to maintain a reduced environment, which favored the survival and differentiation of adult cardiac stem cells [78]. Although the effects of redox modifications of these transcription factors upon resveratrol treatment remain largely unknown, these studies suggest the possibility of manipulating stem cell differentiation by antioxidant drug treatment.

4. The role of kinases in redox regulation of stem cell self-renewal and differentiation

In addition to transcription factors, several kinases involved in ROS signaling or cell cycle also modulate the exquisite balance of stem cell self-renewal and differentiation (Fig. 3). More importantly, most of these key kinases are regulated by redox modifications (Table 1).

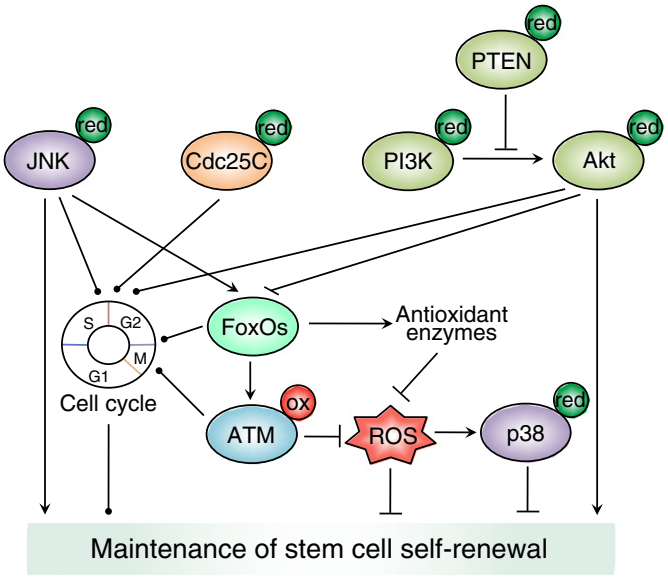

Fig. 3. Kinases in redox regulation of stem cell self-renewal. ROS signaling and cell cycle progression modulated by kinases are crucial in the regulation of stem cell self-renewal and differentiation. JNK, Akt, Cdc25C and ATM, all of which are redox sensors, are implicated in the control of cell cycle. In addition, JNK and Akt can positively or negatively regulate the transcriptional activity of FoxOs, respectively. FoxOs are able to activate the expression of antioxidant enzymes or elevate the activity of ATM to reduce intracellular ROS levels, thereby regulating the self-renewal of stem cells. Moreover, p38, another kinase recognized as a redox sensor, can regulate stem cell self-renewal in response to oxidative stress. 'Ox' denotes the oxidized state, while 'red' denotes the reduced state. Abbreviations: ROS, reactive oxygen species; JNK, c-Jun N-terminal kinase; ATM, ataxia telangiectasia mutated; FoxO4, forkhead box protein O4; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog.

4.1. PI3K/Akt

Phosphoinositide 3-kinase (PI3K)/Akt signaling modulates numerous downstream signaling molecules involved in the regulation of cell survival, cell cycle progression and cell proliferation [79]. Moreover, PI3K/Akt signaling is important for the phosphorylation of FoxOs, by which the nuclear translocation and transcriptional activity of FoxOs are inhibited, leading to inactivation of FoxOs-induced antioxidant enzymes [80]. PI3K/Akt signaling can promote self-renewal and suppress differentiation in coordination with Wnt signaling in both HSCs and ESCs [81,82]. In NSCs, PI3K/Akt signaling is required for ROS-regulated self-renewal and neurogenesis [83]. It is reported that both PI3K and Akt kinases are susceptible to oxidation. Leonard and colleagues developed a cell-permeable chemical probe capable of detecting sulfenic acid-modified proteins directly in living cells to profile the 'sulfenome'. They found that the catalytic subunit of PI3K is modified by sulfenic acid [84]. Akt1 can be oxidized by forming sulfonic acid at Cys310 on exposure to H_2O_2 [85]. The analysis of the crystal structure of inactive form of Akt2 shows a disulfide formation in the activation loop [86]. Indeed, in response to H_2O_2 treatment, Akt2 undergoes the formation of a disulfide bond between Cys297 and Cys311, leading to increased dephosphorylation and inactivation of Akt2 [87]. Furthermore, PI3K/Akt signaling can also be regulated by redox switches of phosphatase and tensin homolog (PTEN), the major gatekeeper of PI3K/Akt signaling (Fig. 3). Redox modifications of PTEN by disulfide bond formation between Cys71 and Cys124 or S-nitrosylation of, to date, undefined cysteines potentiate inactivation of PTEN, thereby stimulating Akt signaling [88,89]. A recent study demonstrated that PTEN can be oxidized by NADPH oxidase-mediated H_2O_2 production during acute infection in myeloid progenitor cells. The oxidized PTEN (deactivated PTEN) then stimulates PI3K/Akt signaling and participates in emergency granulopoiesis [90].

4.2. JNK

c-Jun N-terminal kinase (JNK), one subfamily of the mitogen-activated protein kinases (MAPKs), can counteract Akt-mediated inhibition of FoxOs by phosphorylating FoxOs [91]. JNK was reported to induce the differentiation of mouse ESCs by activating a novel class of target genes [92]. Another study on ISCs suggests that JNK contributes to the loss of redox homeostasis by inducing proliferation and promoting the accumulation of misdifferentiated ISC daughter cells [93]. These results indicate that JNK is required for the regulation of stem cell differentiation. Like PTEN, JNK is also a redox sensor that can be S-nitrosylated. The S-nitrosylation of Cys116 leads to profound inhibition of phosphorylation and subsequent inactivation of JNK1 [94].

4.3. p38

In addition to JNK, p38, which is another member of the MAPK family, is also a redox sensor that modulates stem cell self-renewal and differentiation. Using thiol-affinity chromatography with activated thiol affinity beads (called Purification of Reversibly Oxidized Proteins (PROP)), Templeton and coworkers found that all four cysteines of p38 (Cys39, Cys119, Cys162, Cys211) can be oxidized by H_2O_2, resulting in the loss of p38 kinase activity [95]. Recently, increasing evidence has also demonstrated that p38 is a key regulator that modulates self-renewal and differentiation in several types of stem cells, including HSCs [96], ESCs [97] and NSCs [98]. Somewhat surprisingly, p38 activation in the regulation of self-renewal and differentiation in these stem cells is always stimulated by ROS [96–98].

4.4. ATM

Ataxia telangiectasia mutated (ATM), which can activate a key cell cycle checkpoint in response to DNA damage as well as control the

intracellular ROS levels [99,100], is another kinase that can be both a regulator of stem cell self-renewal and a redox sensor. ATM-mediated inhibition of intracellular ROS levels is required for maintaining self-renewal and the repopulating potential of HSCs [101,102]. Further studies suggest that FoxO3-repressed ROS, that is required for maintenance of the HSC pool, is in part due to the regulation of ATM by FoxO3 [27]. As a redox sensor, ATM can be oxidized at Cys2991 to form a disulfide-cross-linked homodimer following exposure to H_2O_2, resulting in ATM inactivation [103,104].

4.5. Cdc25

Since cell cycle regulation is crucial for the balance of stem cell self-renewal and differentiation [13], the cyclin-dependent kinase (Cdk) complexes should be involved. This is partially supported by the fact that ESCs have high levels of constitutively active Cdk2-cyclin A/cyclin E activity to avoid the early G1 phase of the cell cycle and achieve the goal of self-renewal [13,105]. The Cdc25 phosphatases are essential for cell-cycle progression and checkpoint control by activating Cdk complexes (Cdk1-cyclin A/cyclin B, cyclin E-Cdk2) for M phase entry [106]. In addition, Cdc25 is shown to be crucial for stem cell maintenance and proliferation of germline stem cells and cyst stem cells in *Drosophila* testis [107]. It has been shown that H_2O_2 treatment is able to stimulate the formation of an intracellular disulfide bond between Cys330 and Cys377 in Cdc25C, leading to Cdc25C degradation [108], suggesting that oxidization-mediated Cdc25C might be involved in Cdc25-regulated stem cell self-renewal.

5. The role of microRNAs in redox regulation of stem cell self-renewal and differentiation

MicroRNAs, a group of small noncoding RNAs, are important versatile regulators of gene expression which modulate the translation and degradation of mRNAs [109]. MicroRNAs play key roles in regulating cell fate including stem cell self-renewal and differentiation by suppressing a range of target genes [110]. Indeed, upregulation of miR10b and miR23b induced by Argonaute 2 silencing was able to induce apoptosis of adipose tissue-derived stem cell (ATSC) *via* ROS-mediated p38 MAPK inactivation, leading to reduced self-renewal. Further studies indicated that the elevated level of ROS caused by miR10b and miR23b was due to the inhibition of antioxidant genes, including TXNL2 (also termed Grx3) and GPx3 [111]. In another study, miR210 was found to alleviate oxidative stress to protect MSCs from apoptosis, which was partially reversed by c-Met pathway repression [112]. These studies imply that regulation of stem cell fate by miRNAs is dependent on their distinct targets (Fig. 4). However, whether miRNAs regulate ROS induce stem cell apoptosis *via* redox modifications of key proteins still remains to be demonstrated.

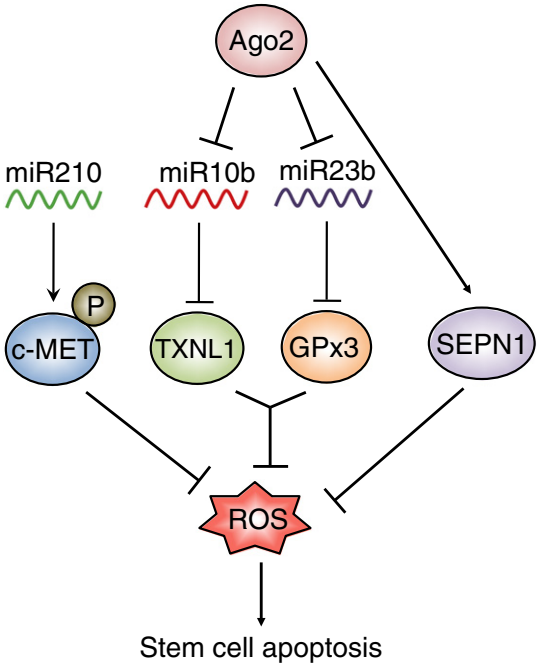

Fig. 4. MicroRNAs in redox regulation of stem cell fate. MicroRNAs have a dual role in redox regulation of stem cell fate due to their distinct targets with different functions.

6. The clinical relevance of modulating redox status in stem cells

The redox status of stem cells is an important limiting factor for stem cell differentiation [113,114]. Studies have revealed a relative higher level of endogenous superoxide in differentiated stem cells [74]. Treatment with exogenous superoxide (such as H_2O_2) can stimulate the differentiation of stem cells, which can be reversed by the H_2O_2 scavenger NAC [10]. In addition, as an important regulator and indicator of intracellular redox status, GSH redox potential is closely related with stem cell differentiation: on one hand, stem cells (including embryonic stem cells and iPS cells) have relatively low levels of cystine transporter (xCT), leading to increased level of intracellular GSH [115]; on the other hand, stem cells with more oxidized GSH will decrease proliferation and initiate differentiation [116]. Although the ROS-mediated redox status of stem cells play an important role in stem cell differentiation, and much work has been done trying to explain how ROS signaling regulates stem cell differentiation, most of these studies have ignored the fact that ROS may cause redox modifications of key proteins (including transcriptional factors and kinases) and change, or even reverse, their functions compared with reduced forms of these proteins. Although this new era of redox-related studies have been investigated in tumor biology, little work has been done in stem cells until now. A recent study by Luo's group has demonstrated that ROS can oxidize and deactivate PTEN, which then upregulates the PI3K/Akt pathway in bone marrow myeloid progenitors, resulting in emergency granulopoiesis [90]. This innovative study supports our hypothesis that redox modifications can modulate the fate of stem cells. Almost certainly the function of more redox sensors in stem cells will be studied in the near future. Given the importance of redox status in the self-renewal and differentiation of stem cells, efforts should be made to find a way to readily monitor the redox status of redox sensors to facilitate the clinical use of stem cells. Chemical intervention with drugs such as NAC [10], resveratrol [78], synthetic condensed 1,4-naphthoquinone derivative [11], and tauroursodeoxycholic acid [117], have been reported to modulate ROS levels to change the redox status of stem cells. A more effective way may, still to be pursued, be to directly target the redox modifications of redox sensors [118] leading to novel ways of treating a range of diseases using stem cells.

7. Conclusions

Much progress has been achieved during the last decade in identifying redox sensors due, in part, to the remarkable development of redox proteomic technology for high throughput profiling. Although redox modifications can switch protein function, most studies have focused on their effect at the individual protein level and less is known about the roles that redox sensors play in the modulation of integrative signaling networks and biological behavior. A number of transcription factors, kinases and other proteins that play critical roles in regulating stem cell self-renewal and differentiation are now recognized as redox sensors. These important regulators are involved in either ROS signaling or cell cycle control or both. Further understanding of whether redox modifications of these redox sensors are required in the balance of stem cell self-renewal and differentiation will provide novel insight

into stem cell biology, and will enable better maintenance of stem cells isolated from tissues or umbilical cord blood for clinical use.

Abbreviations

ARE	antioxidant responsive element
ATM	ataxia telangiectasia mutated
Cdk	cyclin-dependent kinase
Cys	cysteine
CSCs	cancer stem cells
ESCs	embryonic stem cells
FoxO	forkhead box protein O
hBMSCs	human bone marrow derived mesenchymal stem cells
HIF-1α	hypoxia-inducible factor 1α
IKKβ	IκB kinase
ISCs	intestinal stem cells
JNK	c-Jun N-terminal kinase
Keap1	kelch-like ECH-associated protein 1
LIF	leukemia inhibitory factor
MAPK	mitogen-activated protein kinase
MSCs	mesenchymal stem cells
NAC	N-acetyl-cysteine
NF-κB	nuclear factor kappa B
Nrf2	nuclear factor erythroid-2-related factor 2
NSCs	neural stem cells
PI3K	phosphoinositide 3-kinase
PROP	Purification of Reversibly Oxidized Proteins
PTEN	phosphatase and tensin homolog
Ref-1	redox factor-1
RNS	reactive nitrogen species
ROS	reactive oxygen species
R-SH	thiol
R-SNO	S-nitrosothiol
R-SNO_2	S-nitro-thiol
R-SO_2H	sulfinic acid
R-SO_3H	sulfonic acid
R-SOH	sulfenic acid
R–S–S–G	S-glutathionylation
R–S–S–R	intracellular disulfide bond
R–S–S–R′	intercellular disulfide bond
SOD	superoxide dismutase
STAT3	signal transducer and activator transcription factor 3

Disclosure of potential conflicts of interest

The authors indicate no potential conflicts of interest.

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