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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.