CURRENT OPINION Oxidation and erythropoiesis

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Purpose of review
Erythropoiesis is a complex multistep process going from committed erythroid progenitors to mature red cells. Although recent advances allow the characterization of some components of erythropoiesis, much still remains to be investigated particularly on stress erythropoiesis. This review summarizes recent progresses made to understand the impact of oxidative stress on normal and pathologic erythropoiesis.

Recent findings
During erythroid maturation, reactive oxygen species might function as second messenger through either transient oxidation of cysteine residues on signaling targets or modulation of intracellular signaling pathways. Thus, in erythropoiesis, efficient cytoprotective systems are required to limit possible reactive oxygen species-related toxic effects especially in stress erythropoiesis characterized by severe oxidation such as β-thalassemia. In addition, prolonged or severe oxidative stress impairs autophagy, which might contribute to the block of erythroid maturation in stress erythropoiesis. Understanding the functional role of cytoprotective systems such as peroxiredoxin-2 or classical molecular chaperones such as the heat shock proteins will contribute to develop innovative therapeutic strategies for ineffective erythropoiesis.

Summary
We provide an update on cytoprotective mechanisms against oxidation in normal and stress erythropoiesis. We discuss the role of oxidative sensors involved in modulation of intracellular signaling during erythroid maturation process in normal and stress erythropoiesis.

Keywords
autophagy, erythropoiesis, forkhead-box-calls-O3, kinase, Nfr2, thalassemia

INTRODUCTION

Erythropoiesis is a complex multistep process going from committed erythroid progenitors to mature red cells. Although recent advances allow the characterization of some components of erythropoiesis, much still remains to be investigated particularly on stress erythropoiesis.

Erythroid differentiation is characterized by the production of reactive oxygen species (ROS) both in response to erythropoietin (EPO) and to the large amount of iron imported into the cells during heme biosynthesis coordinated with α/β-globin chain synthesis. In erythropoiesis, ROS might also function as second messenger through either transient oxidation of cysteine residues on signaling targets or modulation of signaling pathways mainly involving kinases [1–4,5■,6,7]. Thus, in erythropoiesis, efficient cytoprotective systems are required to limit possible ROS-related toxic effects especially in stress erythropoiesis characterized by severe oxidative stress such as β-thalassemia [8–13]. In the present review, we focus on cytoprotective and antioxidant systems in normal and stress erythropoiesis, summarizing the recent advancement on the mechanisms underlying the modulation of signal transduction pathways to assist erythroid growth and maturation.

ANTIOXIDANT AND CYTOPROTECTIVE SYSTEMS IN ERYTHROPOIESIS

The studies of normal and stress erythropoiesis have led to the identification of the key role of cytoprotective and antioxidant systems during erythroid maturation. The importance of controlling ROS generation during erythropoiesis is also supported by the hematological phenotype of mice genetically lacking cytoprotective or antioxidant systems such

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KEY POINTS

- ROS might function as second messenger during erythropoiesis.

- Cytoprotective and antioxidant systems such as peroxiredoxin-2 play a key role against oxidation to ensure erythroid maturation.

- Impaired autophagy might contribute to amplify oxidative stress in pathologic erythropoiesis. Future studies should clarify the possible impact of autophagy activating agent in assisting ineffective erythropoiesis.

as peroxiredoxin 2 (Prx2, Prx2$^{-/-}$ mice) [7,14■■,15–18]. Prx2 has been first described in red cell and represents the third most abundant red cell cytoplasmic protein, which is able to reduce and detoxify a vast range of organic peroxides, H_2O_2, and peroxynitrite [16,19–21]. Prx2$^{-/-}$ mice display chronic hemolytic anemia, associated with ineffective erythropoiesis and oxidative DNA damage similar to that observed in β-thalassemia (Fig. 1) [14■■,15,22]. Recent evidence show that Prx2 expression is modulated during normal erythroid maturation and it is upregulated in β-thalassemia [2,7,15,23]. Indeed, Prx2 serves as both antioxidant

and cytoprotective system, by specific binding to free heme with decrease Prx2 peroxidase activity (Fig. 2) [24]. This is extremely important in stress erythropoiesis such as in β-thalassemia, which is characterized by high levels of ROS and free heme (Fig. 2) [7]. To further investigate the role of Prx2 in stress erythropoiesis, we generated a mouse model genetically lacking Prx2 in the context of thalassemic background (Prx2$^{-/-}$ Hbb$^{3th/+}$) [15]. In Prx2$^{-/-}$ Hbb$^{3th/+}$ mice, the absence of Prx2 worsens the hematologic phenotype of β-thalassemic mice, by amplifying erythroid oxidative stress and ineffective erythropoiesis. This results in activation of the redox-sensitive transcriptional factor nuclear factor erythroid derived 2 (Nfr2), which promotes the upregulation of anti-oxidant responsive elements (ARE)-genes required to ensure cell survival. Thus, in stress or pathologic erythropoiesis, Prx2 and Nfr2 might cooperate to minimize cellular oxidative damage. As a proof-of-concept, we administered the recombinant PEP1-Prx2 fusion protein to Hbb$^{3th/+}$ mice [14■■,15,25,26]. Treatment with PEP1-Prx2 improves anemia of β-thalassemic mice and decreases the extent of liver and spleen iron overload, which is related to chronic hemolytic anemia of β-thalassemia. Our data on PEP1-Prx2 suggest that the potentiation of endogenous
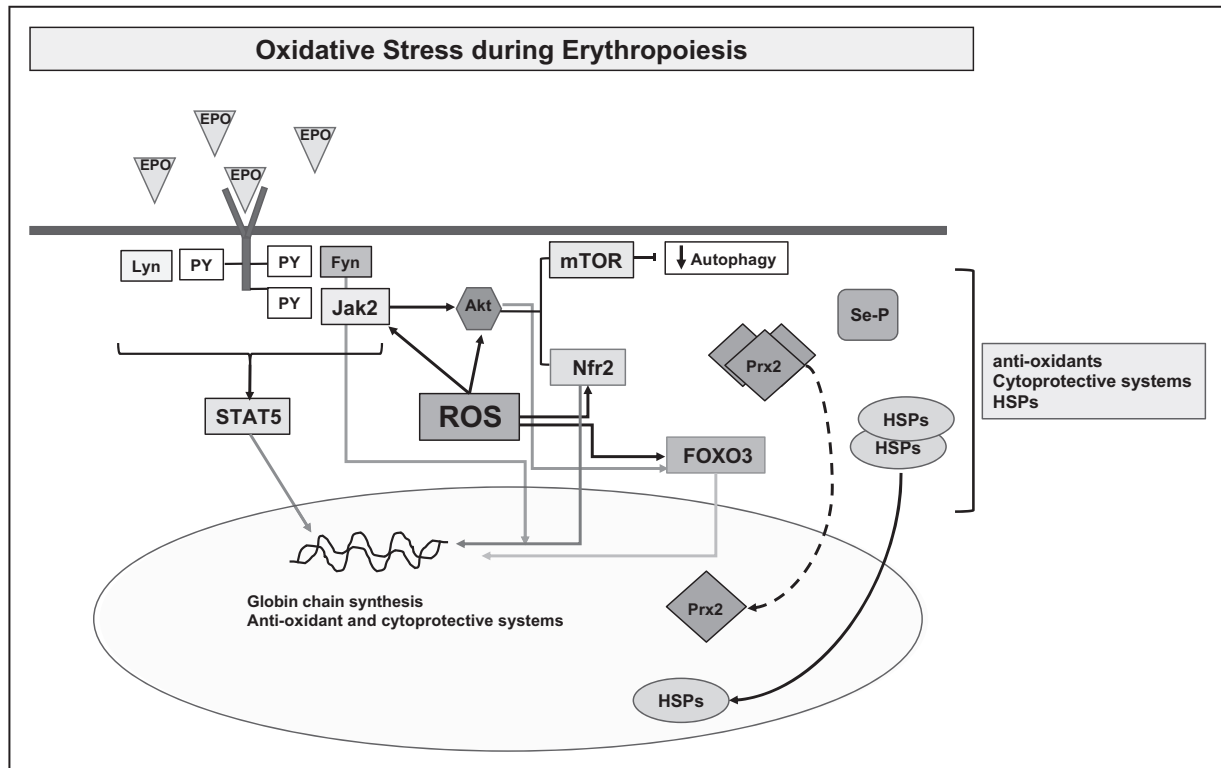

FIGURE 1. Schematic diagram of the effects of oxidative stress during normal erythropoiesis. Akt, Ak strain transforming; EPO, erythropoietin; HSP, heat shock proteins; Jak 2, janus kinase 2; PRX2, peroxiredoxin-2; PY, phospho-tyrosine; ROS, reactive oxygen species; SE-P, seleno protein; STAT5, signal transducer and activator of transcription.
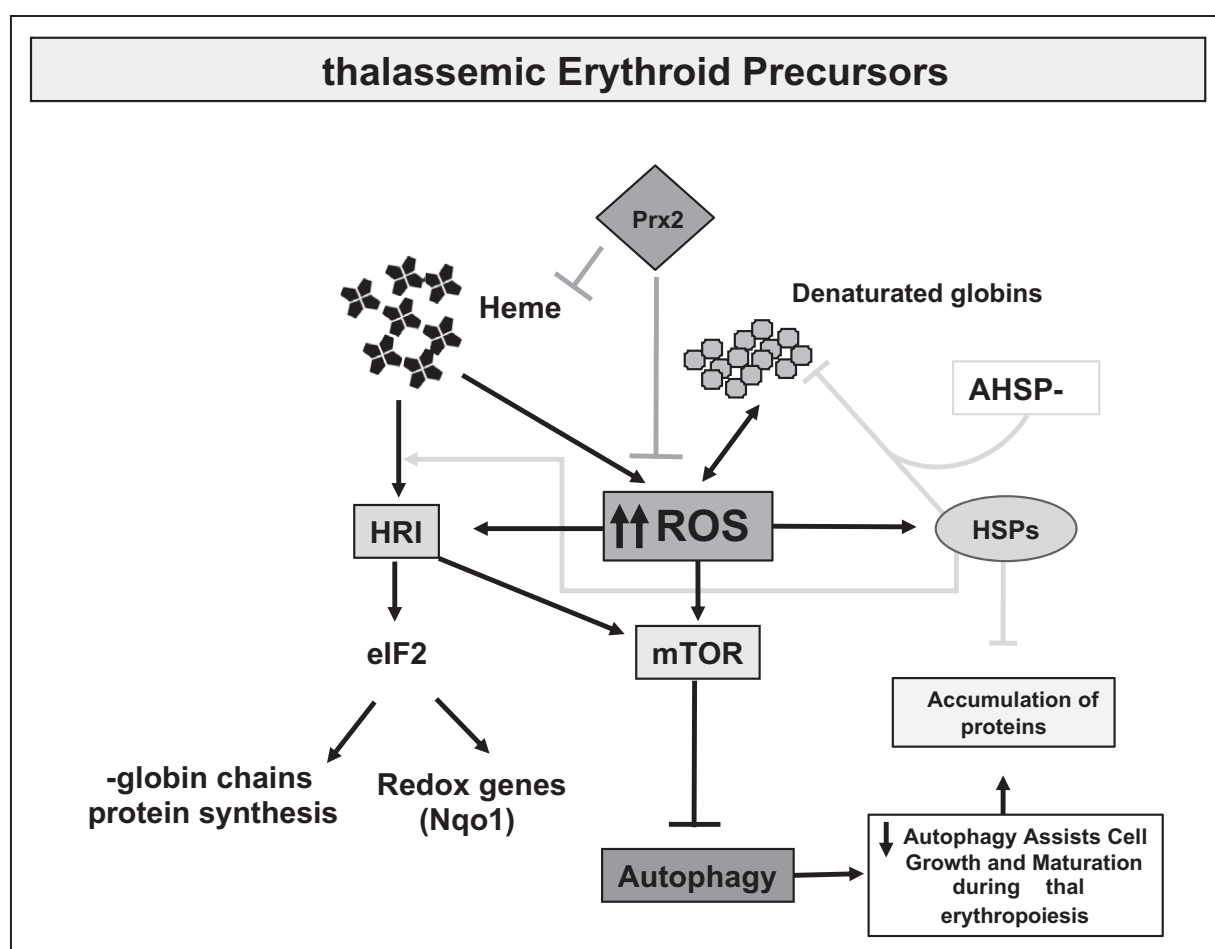
FIGURE 2. Schematic model of the effects of oxidative stress in β-thalassemic erythroid precursors. AHSP-alpha, alpha hemoglobin stabilizing protein-alpha; eIF2-alpha, eukaryotic translation initiation factor 2 alpha kinase; HRI, heme regulated eIF2alpha kinase; HSPs, heat shock proteins; mTOR, mammalian target of rapamycin; Nqo1, NAD(P)H quinone dehydrogenase 1; PRX2, peroxiredoxin-2; ROS, reactive oxygen species.

antioxidant system(s) might represent a new therapeutic strategy against oxidation in pathologic erythropoiesis.

The role of Prx2 as redox-switch protein during erythroid maturation is further supported by the appearance of severe anemia with worsening of ineffective erythropoiesis in Prx2$^{-/-}$ mice treated with iron supplementation to induce iron overload [14"]. In iron-overloaded Prx2$^{-/-}$ mice, we documented a loss of the functional connection between erythroferrone and hepcidin [4,27–29], linking erythropoiesis to iron homeostasis. In addition to target erythropoiesis, Prx2 acts as an on–off switch of signal transducer and activator of transcription (STAT3) transcription activity [30,31], deeply affecting hepcidin expression in response to iron overload. The improvement of both iron-overload-induced ineffective erythropoiesis and liver cytotoxicity by PEP1-Prx2 treatment supports the role of Prx2 as a 'big brother' in optimizing functional pathways linking erythropoiesis to iron homeostasis.

Among the antioxidant systems important in erythropoiesis, the Seleno (Se-) proteins have been described to be required during erythroid maturation events [32–34]. Recent evidence in mice exposed to Se-deficient diet or carrying mutation of gene *Trsp* that controls the synthesis of Se-proteins further support the importance of Se-proteins in normal and stress erythropoiesis [35",36].

In addition to antioxidant systems, heat shock proteins (HSPs) have been also described to assist normal and stress erythropoiesis (Fig. 1) [37–41]. HSP27, 70, and 90 are expressed and modulated during erythroid differentiation and growth. In-vitro cell-based studies have shown that HSP70 and 90 are crucial for the activation of the heme-regulated inhibitor (HRI) of protein translation that represses globin translation in heme-deficient

erythroid precursors of stress erythropoiesis [6,42,43] and the protection of the transcription factor GATA-1 from caspase-3 mediated cleavage [37]. The activation of HRI results in phosphorylation of the α-subunit of eIF2, an important regulatory translation initiating factor, which inhibits the α, β-globin chain synthesis and activates the Atf4 pathway toward redox genes such as heme-oxygenase-1 (ho-1) glutathione S-transferase, and NAD(P)H quinone oxidoreductase 1 (Nqo1) (Fig. 2) [6,44]. This might favorably impact pathologic erythropoiesis such as β-thalassemic ineffective erythropoiesis. The importance of HSP70 nuclear translocation in protecting GATA-1 during erythroid maturation is supported by evidences in β-thalassemic erythropoiesis, in which the large part of HSP70 binds to cytoplasmic free α-chains (Figs. 1 and 2). This markedly reduces GATA-1 protection, contributing to the block of the terminal phase of β-thalassemic erythroid maturation [38].

Another cytoprotective system first described in β-thalassemic erythropoiesis is the α-hemoglobin-stabilizing protein (AHSP). AHSP acts by binding heme-free or heme-replete free α-globin chains, stabilizing their structure and inhibiting β-globin expression (Fig. 2) [45–48]. Indeed, anemia of β-thalassemic mice is more severe in β-thalassemic/AHSP-deficient mice [45–48]. The impact of abnormalities of AHSP in β-thalassemia patients is still under evaluation, the link between decreased AHSP expression and severity of β-thalassemic syndromes remains speculative [49,50].

The tight control of the redox balance during erythropoiesis also involves the redox-sensitive transcription factor Forkhead-box-calls-O3 (FOXO3), which controls several scavenging enzymes such as catalase or glutathione S-transferase [7,10,11,51,52]. In in-vitro model of human β-thalassemic erythropoiesis, we recently show that activation of FOXO3 by resveratrol, a polyphenolic-stilbene, upregulates antioxidant systems, enabling pathologic erythroid precursors to resist to oxidative stress [7]. Thus, modulation antioxidant systems by potentiation of endogenous antioxidants (i.e., PEP-Prx2 or FOXO3 activators) or by exogenous antioxidant molecules (resveratrol or quercetine) might be considered as potential novel therapeutic strategy in treating ineffective erythropoiesis [7,53–55].

REDOX-SENSITIVE SIGNAL TRANSDUCTION PATHWAYS IN ERYTHROPOIESIS

In the last decade, progresses have been made on the characterization of signal transduction pathways involved in erythropoiesis. ROS increases in response to EPO, activating the primary kinase, janus kinase 2 (Jak2), and the secondary kinases, Lyn (proto-oncogene Lyn) and Fyn (proto-oncogene Fyn) [5■,9,56–63]. These kinases also target STAT5 transcriptional factor, harmonizing the EPO-induced signaling cascade and the erythroid maturation events. The importance of both Lyn and Fyn Tyrosine (Tyr)-kinases is supported by model of stress erythropoiesis, showing the persistence anemia in mice genetically lacking either Lyn (Lyn$^{-/-}$) or Fyn (Fyn$^{-/-}$) kinases treated with phenylhydrazine [5■,59,62].

Fyn kinase has been also described to be involved as downstream regulator of the redox-sensitive transcriptional factor Nfr2 in different cell-based systems [64–66]. We recently show that the absence of Fyn results in persistent activation of Nfr2 and cytoplasmic accumulation of nonfunctional, damaged proteins because of impairment of autophagy during erythropoiesis [5■]. This further amplifies intracellular oxidative stress, resulting in dyserythropoiesis with detrimental effect on Fyn$^{-/-}$ erythroid maturation. In Fyn$^{-/-}$ mice, increased ROS promotes overactivation of Jak2, resulting in increased Akt (Ak strain transforming kinase) phosphorylation state and activation of mammalian target of rapamycin (mTOR), the gatekeeper of autophagy [5■]. In Fyn$^{-/-}$ mouse erythroblasts, mTOR signaling blocks autophagy with accumulation of nonfunction/damaged proteins, which further amplified oxidation with severe cytotoxic effect. Noteworthy, overactivation of Ja2-Akt-mTOR pathway has been also reported in mice genetically lacking the redox-sensitive transcriptional factor FOXO3, which are characterized by ineffective erythropoiesis similar to β-thalassemia [51,58,67]. Growing evidence in erythropoiesis suggest that the Serin (Ser-) Threonin (Threo-) kinase, Akt, intersects different signaling pathways, against oxidation or involved in cell growth, differentiation or cell metabolism [58,68]. This latter is mainly driven by the phosphatidylinositol-4, 5-bisphosphate 3 kinase (PI3K)/Akt pathway, regulating the synthesis of 1, 3-bisphosphoglycerate that is part of the cell machinery for glycolysis during erythropoiesis [10,58,69,70].

Among the Ser-Threo kinases linked to EPO cascade and sensitive to oxidation, the extracellular signal-regulated kinase (Erk)-1 and 2 have been involved in cell proliferation events with negative role in cellular differentiation in the early phase of erythropoiesis [68,71]. Studies in-vitro β-thalassemic erythropoiesis have shown activation of Erk1/2 kinases, which may possibly act toward Bcl2 associated X protein/B cell lymphoma 2 system, promoting either proliferation in early sate of β-

thalassemic erythroid differentiation or apoptosis of β-thalassemic erythroid precursors in the late phase of erythropoiesis [68,71].

Taken together these studies point out the importance of redox modulation of signaling pathways, which are involved in cell maturation and differentiation to ensure cell survival and support erythropoiesis against oxidation.

OXIDATION INDUCES ACTIVATION OF AUTOPHAGY TO ASSIST ERYTHROID MATURATION

Autophagy is involved in quality control processes during erythroid maturation, allowing the clearance of unfolded damaged proteins as well as consume organelles [5■,44,58,72,73■■,74–76,77■■,78]. Oxidation activates autophagy to ensure the development and conclusion of erythroid maturation events. However, an intense or prolonged oxidative stress overwhelming autophagy, culminates into cell apoptosis and/or block in cell maturation (Fig. 1). Recently, we show an impairment of autophagy with perturbation of erythropoiesis and increased oxidation in in-vitro model of human erythropoiesis derived from CD34+ cells of patients with chorea-acanthocytosis, a rare neurodegenerative disease, involving also erythroid cells [73■■]. Similar findings have been also reported in iron-deficient erythropoiesis [44]. In β-thalassemic erythropoiesis, the severe intracellular oxidation results in activation of Akt-mTOR pathway, repressing autophagy and triggering apoptosis (Fig. 2) [73■■]. This is also

the case of ineffective erythropoiesis of mice genetically lacking Fyn [5■].

The importance of autophagy in assisting erythropoiesis is further support by evidence in different mouse models of stress erythropoiesis treated with mTOR inhibitors such as rapamycin or sirolimus [5■,58,75,79,80]. Table 1 summarizes the more relevant studies on the impact of mTOR inhibitor(s) either on normal erythropoiesis or phenylhydrazine-induced stress erythropoiesis or ineffective erythropoiesis [5■,58,75,79,81,82]. Noteworthy, in normal erythropoiesis, the inhibition of mTOR results in worsening erythropoiesis as also supported in mouse model genetically lacking mTOR [81]. Otherwise, in model of ineffective erythropoiesis associated with increased ROS and impaired autophagy, the pharmacologic inhibition of mTOR activates autophagy, which assist cell growth and differentiation. This indicates the importance of control intracellular oxidation to ensure efficient autophagy as adaptive mechanism to stressful conditions. In erythropoiesis, the prolonged or severe oxidative stress promotes autophagy dysfunction, amplifying intracellular oxidative damage and triggering cell apoptosis. Thus, agents modulating autophagy in pathologic erythropoiesis might represent a new interesting strategy to improve ineffective erythropoiesis.

CONCLUSION

The mechanisms involved in controlling oxidation during normal and pathologic erythropoiesis are

Table 1. Effects of mTOR inhibitors on murine erythropoiesis

Model	mTOR inhibitors and erythropoiesis	Reference
WT mice	*Torin 1:* potent, selective ATP competitive inhibitor of mTOR Torin 1-induced ineffective erythropoiesis	Guo F *et al.* 2013 [81]
WT mice PHZ-induced stress erythropoiesis	*Rapamycin or sirolimus:* mTOR inhibitor; *MLN0128 or sapanisertib:* second-generation ATP competitive pan-mTOR inhibitor PHZ-treated mice showed prolonged anemia (Rapamycin) and increased mortality (MLN0128)	Knight *et al.* 2014 [75]
Fyn$^{-/-}$ mice Increased ROS and dyserythropoiesis	*Rapamycin or sirolimus:* mTOR inhibitor - Rapamycin ameliorated Fyn$^{-/-}$ mouse dyserythropoiesis - Rapamycin restored physiologic hematologic response to PHZ-treatment in Fyn$^{-/-}$ mice	Beneduce *et al.* 2018 [5■]
β-thalassemic mice Increased ROS and ineffective erythropoiesis	*Rapamycin or sirolimus:* mTOR inhibitor Rapamycin ameliorated β-thalassemic ineffective erythropoiesis and improved anemia of β-thalassemic mice	Zhang *et al.* 2014 [58]
SCD Expanded erythropoiesis with limited ineffective erythropoiesis	*Rapamycin or sirolimus:* mTOR inhibitor Rapamycin supported SCD erythropoiesis and ameliorated the quality control process of terminal phase of erythroid maturation, improving sickle red cell features	Wang J *et al.* 2016 [79]

PHZ, phenylhydrazine; ROS, reactive oxygen species; SCD, sickle cell disease; WT, wild-type.

still far from being fully understood. However, progresses have been made in characterization of the functional role of antioxidant systems and cytoprotective system such as Prx2 of HSP70 during erythropoiesis. The intense cross-talk between different cellular functional compartments and the dynamic cellular changes during erythropoiesis highlights the importance of a tight control of ROS levels by antioxidant and cytoprotective systems, which also interface crucial signal transduction pathways involving Jak2, Fyn, or Akt. An impairment of autophagy further contributes to oxidative stress sustained by ineffective erythropoiesis. Thus, the beneficial effects of the inhibitors of the gatekeeper of autophagy, mTOR, indicates that optimization of the quality control processes might support ineffective erythropoiesis, ensuring erythroid maturation and growth.

Further studies need to be carried out to understand the role of cytoprotective systems during normal and pathologic erythropoiesis. In view of the specific and characteristic association of ROS with ineffective erythropoiesis, cytoprotective, and antioxidant systems constitute an interesting research target.

Conflicts of interest

There are no conflicts of interest.

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- ■■ of outstanding interest

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