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Reactive oxygen species: Orchestrating the delicate dance of platelet life and death

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ABSTRACT

Platelets, which are vital for blood clotting and immunity, need to maintain a delicately balanced relationship between generation and destruction. Recent studies have highlighted that reactive oxygen species (ROS), which act as second messengers in crucial signaling pathways, are crucial players in this dance. This review explores the intricate connection between ROS and platelets, highlighting their dual nature. Moderate ROS levels act as potent activators, promoting megakaryocyte (MK) differentiation, platelet production, and function. They enhance platelet binding to collagen, increase coagulation, and directly trigger cascades for thrombus formation. However, this intricate role harbors a double-edged sword. Excessive ROS unleash its destructive potential, triggering apoptosis and reducing the lifespan of platelets. High levels can damage stem cells and disrupt vital redox-dependent signaling, whereas uncontrolled activation promotes inappropriate clotting, leading to thrombosis. Maintaining a precise balance of ROS within the hematopoietic microenvironment is paramount for optimal platelet homeostasis. While significant progress has been made, unanswered questions remain concerning specific ROS signaling pathways and their impact on platelet disorders. Addressing these questions holds the key to unlocking the full potential of ROS-based therapies for treating platelet-related diseases such as thrombocytopenia and thrombosis. This review aims to contribute to this ongoing dialog and inspire further exploration of this exciting field, paving the way for novel therapeutic strategies that harness the benefits of ROS while mitigating their dangers.

Abbreviations

AHR	aromatic hydrocarbon receptor
AP-1	Activator protein 1
Apaf-1	Apoptotic protease activating factor-1
CAT	Catalase
c-Kit	CD117
CMPs	Common bone marrow progenitor cells
CYP450	Cytochrome p450
DOUX	Dual oxidase
DPI	Diphenyleneiodonium chloride

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ECM	Extracellular matrix
EGF	Epidermal growth factor
ER	Endoplasmic reticulum
ETC	Electron transport chain
G-CSF	Granulocyte colony stimulating factor
GM-CSF	Granulocyte macrophage colony stimulating factor
GP	Glycoprotein
GSH	Glutathione
GSH-EE	Glutathione ethyl ester
H_2O_2	Hydrogen peroxide
HSC	Hematopoietic stem cell
IL-3	Interleukin-3
IL-6	Interleukin-6
JNK/SAPK	c-Jun N-terminal kinase/Stress-activated protein kinase
MK	Megakaryocyte
MEP	Megakaryocyte erythroid progenitor cell
mTOR	Mechanistic target of rapamycin
NAC	N-acetylcysteine
NADPH	Nicotinamide adenine dinucleotide phosphate
NF-E2	Nuclear factor erythroid 2
NF-κB	Nuclear factor kappa-B
NOX	NADPH oxidase
Nrf2	Nuclear factor erythroid 2-related factor 2
O•2-	Superoxide anion
•OH	hydroxyl
OH−	hydroxide ion
PDGF	Platelet-derived growth factor
PKC	Protein kinase
PMA	Phorbol 12-myristate 13-acetate
PPARγ	Peroxisome proliferator activated receptor gamma
ROS	Reactive oxygen species
SOD	Superoxide dismutase
Srf	serum response factor
TGF-β	Transforming growth factor-β
TLR2	Toll-like receptor 2
TLR4	Toll-like receptor 4
TPO	Thrombopoietin
VWF	Von Willebrand Factor
XO	Cytoplasmic xanthine oxidase

1. Introduction

Platelets are tiny, nucleusless blood cells with critical roles in hemostasis, wound healing, vasoconstriction, inflammation and the immune response [1]. Platelet homeostasis refers to the dynamic balance of platelet production and clearance in the body, with the result that the number and function of platelets remain relatively stable [2]. In humans, the normal reference range for platelet count is 150–400 $\times$ 10^9/L blood, and their lifespan is approximately 7–10 days. To maintain a consistent level, the human body must produce 10 $\times$ 10^9 platelets per day [3]. However, dysregulation of platelet production or destruction can disrupt platelet homeostasis, leading to thrombocytopenia, thrombocytosis, increased risk of bleeding or thrombosis, and even death [4]. Thrombocytopenia is a platelet count of less than 150 $\times$ 10^9 per liter of blood. Patients with a platelet count greater than 50 $\times$ 10^9 per liter are generally asymptomatic. Patients with platelet counts between 20 and 50 $\times$ 10^9 per liter may have mild skin manifestations, such as petechiae, purpura, or ecchymosis. Patients with platelet counts of less than 10 $\times$ 10^9 per liter are at high risk of serious bleeding [5]. Therefore, maintaining proper platelet homeostasis is critical for the body.

ROS encompass a group of highly reactive molecules and ions generated in response to both internal and external stimuli. This category includes both free radicals and nonradical oxygen molecules such as •OH, O•2- and H_2O_2 [6]. ROS play crucial roles in regulating normal physiological processes, signal transduction and delicate equilibrium during cell development. These processes involve aspects such as cell cycle progression, proliferation, differentiation, migration, immune responses and programmed cell death [7,8]. Notably, ROS production and levels fluctuate in various diseases and physiological contexts [9]. Nonetheless, under the influence of various factors in the body, ROS levels can increase significantly. An imbalance between ROS formation and antioxidant mechanisms can lead to oxidative stress, causing damage to critical biomolecules such as proteins, lipids, and DNA [10,11]. This oxidative stress is relevant to numerous pathological conditions, including cancer [12,13], diabetes [13], cardiovascular disease [14] and neurodegenerative diseases [15]. ROS influence a broad spectrum of cellular processes across all domains of life, from bacteria and plants to animals, including humans. Consequently, it is clear that ROS have a profound impact on the onset and progression of many diseases, making them potential targets for disease therapy. However, the relationships between the regulation of ROS and platelet generation and lifespan, as well as the temporal and spatial correlates of the regulation of platelet homeostasis by ROS, remain unclear. A systematic and comprehensive review of these elements is still lacking. The regulation of ROS levels is crucial for treating platelet disorders and maintaining the internal environment of an organism.

Over the past few years, increasing evidence has shown that ROS, which serve as secondary carriers in multiple signaling pathways, play essential roles in the regulation of platelet production, platelet function and platelet lifespan [16–18]. Given the potential physiological and pathological ramifications of ROS, modulating ROS levels in a deliberate manner could represent a promising therapeutic approach for maintaining platelet homeostasis. In this work, we correlate platelet homeostasis with ROS and provide a comprehensive review of the dual regulatory role of ROS in platelet production, function and lifespan.

2. Literature search and selection strategy

English-language studies and reviews published from 1994 to 2023 were systematically studied using mainstream databases PubMed and Google Scholar to obtain information related to ROS and platelets. Further literature screening included the following keywords: thrombopoiesis, platelet function, apoptosis, NADPH, mitochondria, oxidative stress and thrombosis. Articles of little relevance, limited content and old age, as well as unpublished reports, were excluded. A total of 365 articles were selected for in-depth analysis of published research in this field (Fig. 1).

3. Mechanisms of ROS generation and clearance in platelets

In platelets, the pathways for ROS generation primarily involve mitochondria, NADPH oxidase, the arachidonic acid metabolic pathway, and the endoplasmic reticulum [19]. Mitochondria are one of the main sources of ROS in platelets. At complexes I and III of the electron transport chain (ETC), electron leakage can lead to the reduction of oxygen to O_2^-, which can subsequently be converted to hydrogen peroxide (H_2O_2) by superoxide dismutase 2 (SOD2) [20,21]. Mitochondria-generated ROS play roles in platelet activation, aggregation, adhesion, and apoptosis and can function as signaling molecules to regulate platelet signal transduction. In platelets, NOX1 and NOX2 from the NADPH oxidase family represent another major pathway for ROS generation. Platelet activators, such as thrombin and collagen, stimulate NOX activity, leading to complex formation with p40phox, p47phox, and p67phox, which increases ROS production and subsequently enhances platelet adhesion and aggregation [22,23]. Platelets contain enzymes such as COX1, which generate peroxides and other ROS during the arachidonic acid metabolic process. It catalyzes the conversion of arachidonic acid to prostaglandin G_2 (PGG_2), a process that produces peroxides and releases ROS, such as H_2O_2 [24]. Additionally, the CYP450 enzyme system in the endoplasmic reticulum generates ROS during oxidative reactions. In CYP450-catalyzed redox reactions, electrons are transferred from NADPH through cofactors (such as P450 reductase) to the CYP450 enzyme, activating the oxygen molecule. During this process, if the redox reaction is incomplete, the partially reduced oxygen molecule can transform into ROS [25,26] (Fig. 2). The

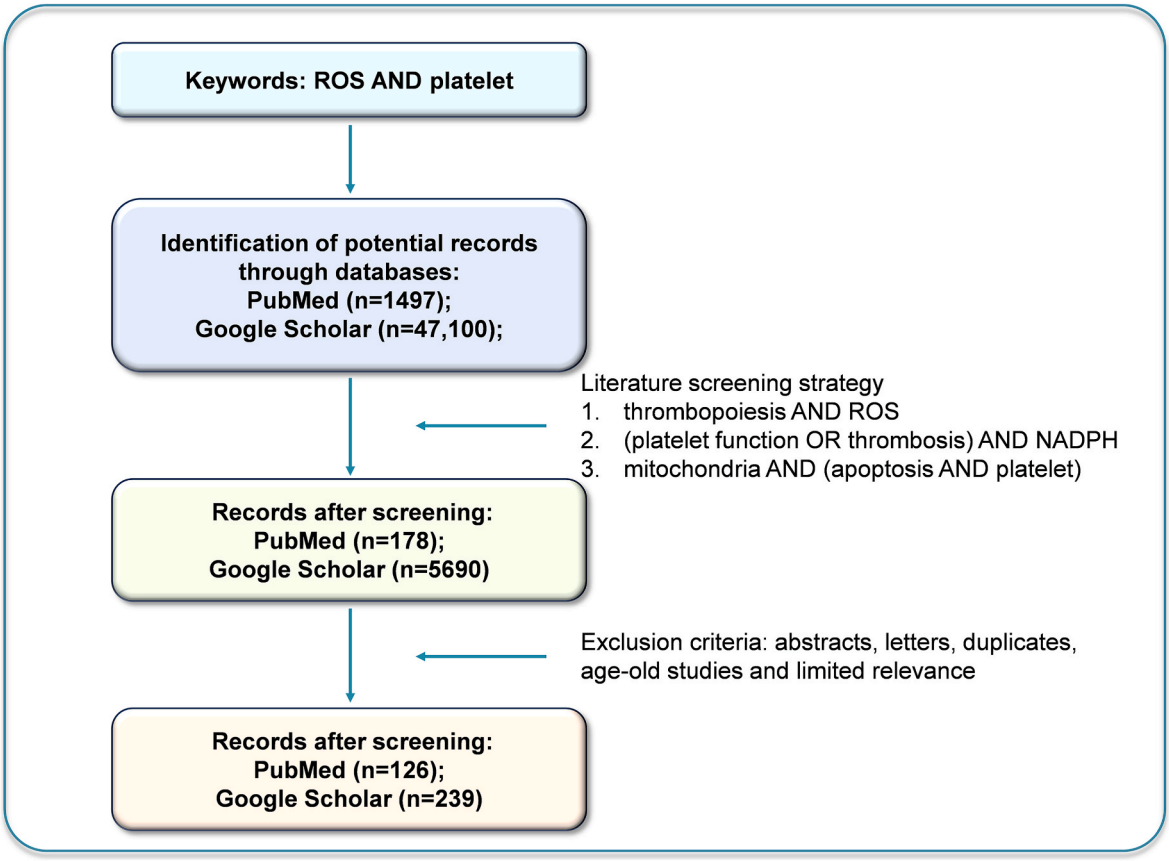

Fig. 1. The PRISMA flowchart illustrates the literature screening process, detailing the strategy and number of articles screened, the number of articles excluded, and the number of articles included in the final analysis. "Age-old studies" refer to studies that were published or completed a long time ago, especially those before the 1960s, which are widely considered old. "Limited relevance" refers to those that are not fully consistent with the research background or content of this review or have little relevance to the issues discussed in this review.

increase in ROS generation in platelets not only plays a crucial regulatory role in platelet activation, aggregation, and adhesion but also exerts significant effects in pathological processes such as coagulation and inflammation.

Antioxidants play a crucial role in maintaining the redox balance in platelets, including SOD, catalase (CAT), and glutathione (GSH) [6]. SOD is one of the most important antioxidant enzymes in platelets, primarily responsible for converting O_2^- into H_2O_2, thereby reducing the direct toxicity of O_2^-. The SOD family consists of three isoforms with different cellular localizations and metal cofactors: the dimeric Cu/Zn-SOD (SOD1), located in the cytoplasm and mitochondrial intermembrane space; the tetrameric Mn-SOD (SOD2), located in the mitochondrial matrix; and the tetrameric Cu/Zn-SOD (SOD3), which is distributed in the extracellular space. Platelets express SOD1 and SOD2. SOD plays an important role in physiological platelet function and the prevention of thrombosis [27]. NAC can be converted to GSH *in vivo*. Together with SOD, GSH helps convert O_2^- into hydrogen peroxide, after which CAT breaks down H_2O_2 into H_2O and O_2, creating a cascade of antioxidant effects [28,29].

In platelets, the dynamic balance between ROS generation and clearance mechanisms is crucial for maintaining normal platelet function. Generated ROS participate in regulating platelet activation and aggregation. However, excessive ROS production can lead to oxidative stress, triggering pathological thrombosis or inflammatory responses. Thus, the antioxidant system removes excess ROS to prevent platelet dysfunction and maintain the body's redox balance.

4. The process of platelet production

In adults, the process of platelet production, known as thrombopoiesis, is a two-stage process involving hematopoietic stem cells (HSCs) differentiating into mature MKs and, subsequently, the release of platelets from these MKs [1]. MKs are rare, large (typically 50–100 μm) cells that account for only approximately 0.05 % of the human bone marrow cell population [30]. During the terminal differentiation of MKs, they undergo a unique process involving intranuclear mitosis, which results in polyploid nuclei, typically averaging 16 N but reaching as high as 128 N. Simultaneously, they create a continuous boundary membrane system (DMS) that interfaces with the plasma membrane, supplying the additional membrane required for platelet formation (Fig. 3) [31]. Once MKs mature, they extend pseudopodia-like protrusions known as pro-platelets into the vascular lumen. Within the lumen, the shear force generated by the flowing blood breaks down the proplatelets into 1–2 μm platelets (Fig. 3) [32]. This process is called platelet production and is powered by the dynamic reorganization of the MK cytoskeleton, particularly involving the sliding and stretching of microtubules [33]. The intricate process of MK development and thrombopoiesis is tightly regulated by multiple cytokines, with TPO playing a pivotal role [31]. Multiple signaling pathways are initiated through TPO and c-Mpl, including the MEK/ERK, PI3K/AKT, JAK2 and STAT3/STAT5 pathways [34–37]. The activation of these pathways modulates the expression of genes specific to MKs, influencing MK development and platelet production [31]. In addition to TPO, several other hematopoietic growth factors with platelet-producing activity exist, including cytokines such as c-Kit ligands and interleukins (IL-1, IL-3, IL-6, and IL-11) [38]. In addition, various transcription factors play crucial roles in regulating

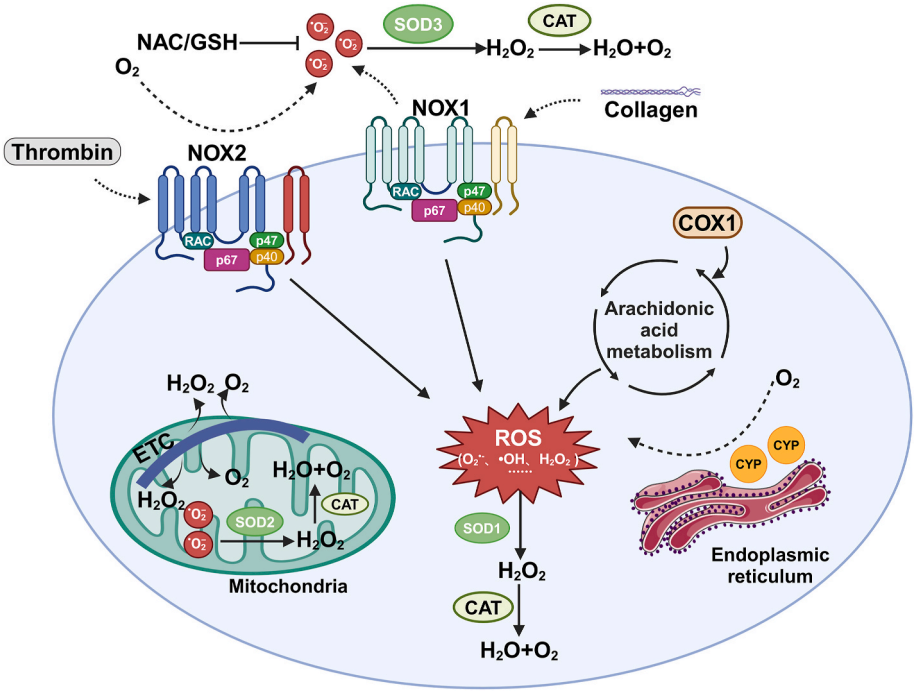

Fig. 2. Summary diagram of ROS generation and removal in platelets. NOX1 and NOX2 are the main ROS-generating enzymes in platelets, mediating the generation of intracellular ROS through the stimulation of collagen and thrombin. As the main organelles for ROS production, mitochondria produce ROS through their electron transport chain, and the endoplasmic reticulum produces ROS through CYP450. Enzymes such as COX1 in platelets produce peroxides and release ROS during the catalysis of arachidonic acid metabolism. Some antioxidant enzymes in cells inhibit the production of excessive ROS to maintain redox balance in the body. Different distributions of SOD1-3 can convert ROS to hydrogen peroxide, which is subsequently decomposed by CAT. NAC can be converted to GSH *in vivo* to play an antioxidant role. ROS generation and scavenging work together to maintain the dynamic balance of ROS in platelets. NOX, NADPH oxidase; SOD, superoxide dismutase; GSH, glutathione; CAT, catalase.

MK terminal maturation and platelet production. These genes include GATA1, RUNX1, NF-E2, GATA1, FOG1, TAL1, FIL1 [39], Srf [40], AHR [41] and the transcription factor ETV6 [42]. Together, these transcription factors and cytokines orchestrate MK maturation and platelet production, playing a vital role in maintaining platelet homeostasis in the body.

5. Positive effects of ROS on thrombopoiesis

ROS are recognized as essential chemical mediators in the regulation of normal hematopoiesis. ROS levels influence a wide spectrum of fundamental physiological processes, ranging from cell differentiation and proliferation to cell death [43]. As research into ROS has progressed, an increasing number of investigators have focused on discerning the impact of ROS levels and oxidative stress on the development and functionality of the hematopoietic system. The hematopoietic microenvironment within the bone marrow is of paramount importance for MK differentiation and maturation [44]. For example, the bone marrow extracellular matrix (ECM) serves as a cell-free component that provides crucial physical support for hematopoiesis. ECM matrix proteins such as VWF, fibrinogen, fibronectin, type IV collagen, and laminin interact with corresponding receptors of MKs, thereby regulating thrombopoiesis [45]. Additionally, extracellular ROS modulate MK differentiation and platelet release in a microenvironment-dependent manner, which is partly driven by increased metabolic activity in HSCs [43]. In a low-oxygen state, $CD34^+$ cells are prevented from entering the differentiation cycle [46], leading to reduced megakaryocyte lineage differentiation. Conversely, higher oxygen levels promote both $CD34^+$ cell division and CD41 expression [47]. Very low ROS levels impair HSC differentiation and self-renewal, hindering repopulation [48]. Altered superoxide in bone marrow stromal cells can also cause improper survival and proliferation of hematopoietic cells in the myeloproliferative abnormal bone marrow microenvironment [49]. During MK differentiation, migration from the osteoblast niche to the vascular niche in the bone marrow is accompanied by changes in the ROS-dependent oxidation state in the bone marrow microenvironment [43]. These results suggest that ROS can modulate HSC activity and influence MK and thrombopoiesis in a bone marrow microenvironment-dependent manner. Additional studies have demonstrated that increased ROS can positively affect MK maturation and platelet release. High oxygen levels near the bone marrow sinusoids and lung capillaries further contribute to platelet release from MKs into the sinusoidal cavity [50]. Notably, ROS also induce $CD34^+$ cell differentiation toward platelets, increase CD41 and CD61 expression, and increase MK ploidy [16]. Overall, these findings suggest that ROS play a significant role in regulating MK development and platelet production.

PMA is a commonly used inducer of MK differentiation. and is associated with the induction of ROS accumulation in MKs. Interestingly, research has shown that dengue virus replication inhibits PMA-induced MK differentiation, reduces the expression of the MK differentiation-associated markers CD41 and CD61, and suppresses ROS accumulation in K562 cells [51]. Additionally, endocannabinoids promote MK differentiation, which is accompanied by MAPK activation and ROS accumulation in Dami cells [52]. Studies have shown that NOX1, which is responsible for ROS production, is highly expressed in primary murine MKs and plays a significant role in promoting ploidy, possibly through the regulation of G1 phase cyclin E [53]. TLR2 is a membrane receptor expressed on MKs and platelets. When it interacts with the specific ligand Pam3CSK4, it induces the activation of several signaling pathways, including the NF-κB, PI3K/Akt and ERK-MAPK pathways. These pathways, in turn, induce the expression of various transcription factors, such as GATA-1, NF-E2 and mTOR, ultimately leading to MK maturation. Furthermore, Pam3CSK4 stimulation results in an increase in the levels of ROS, suggesting that ROS production may also play a role

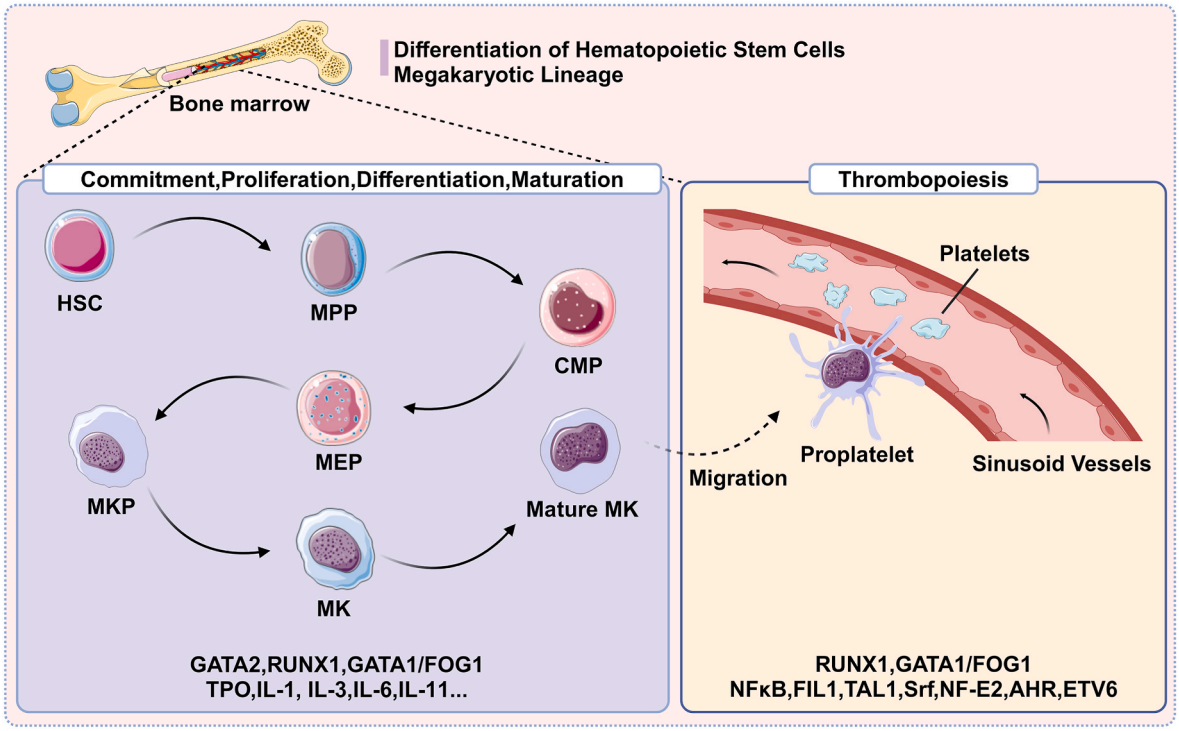

Fig. 3. Platelet production summary chart. These cells initially differentiate from hematopoietic stem cells into pluripotent progenitors, common myeloid progenitors, megakaryotic erythroid progenitors and megakaryotic progenitors, respectively, and then into MKs. MKs undergo intranuclear mitosis, polyploidization and the formation of an invaginated membrane system (IMS) to become mature MKs. The cytoplasm of mature MKs then undergoes reorganization to form extensions known as proplatelets, which migrate to vascular ecological niches in the bone marrow and eventually release platelets into the circulation in response to blood shear forces. TPO is a key regulator of platelet production. Other regulators include the interleukin family. In addition, many transcription factors, such as GATA-1, NF-E2, RUNX1, and TAL1, are involved in regulating various stages of this process. HSCs, hematopoietic stem cells; MPPs, multipotent progenitor cells; CMPs, common myeloid progenitor cells; MEPs, megakaryocytic erythroid progenitor cells; MKPs, megakaryocytic progenitor cells.

in MK maturation.

While the studies mentioned above do not provide direct evidence that ROS promote MK differentiation and maturation, they do imply a positive correlation between ROS levels and the process of MK development. Another compound, 15-deoxy-$\Delta^{12,14}$ prostaglandin J2 (15d-PGJ2), which is a crucial lipid mediator and a peroxisome proliferator–activated receptor gamma (PPARγ) ligand, has been shown to induce MK maturation and platelet production in several cell lines, including Meg-01, M07e and Dami cells and culture-derived mouse and human MKs. Additionally, it stimulates the recovery of platelet levels in mice exposed to ionizing radiation. In terms of its mechanism of action, 15d-PGJ2 facilitates platelet production through the accumulation of ROS, independent of PPARγ. The use of antioxidants such as glutathione ethyl ester (GSH-EE) and NAC can suppress 15d-PGJ2-induced platelet production [16]. A recent study linked ROS accumulation to enhanced MK differentiation and the expression of MK and the platelet markers CD41 and CD61 [51]. Notably, NOX1 was identified as the primary NOX subtype in mouse MKs, playing a key role in ROS production within $CD41^+$ MKs and driving their polyploidization. This generated ROS not only increased MK-related signal transduction and gene expression but also increased MK ploidy *in vivo*. Moreover, ROS significantly influence MK cycle development by modulating G1 cell cycle protein expression and G1-S cell cycle progression [54].In addition to their impact on the aforementioned hematopoietic cells, ROS further govern the fate of common bone marrow progenitor cells (CMPs) by influencing their differentiation potential. Importantly, ROS-depleted CMPs exhibit a heightened propensity to differentiate into MEPs. Strikingly, the ROS level within CMPs was inversely related to the differentiation capacity of MEP colonies. Consequently, higher ROS levels not only promote CSF1R expression but also drive CMPs toward granulocyte–macrophage progenitor cell (GMP) differentiation, significantly suppressing the formation of MEP colonies [55]. These findings illuminate the critical role of ROS in fostering MK development and platelet production (Fig. 4).

6. Mechanisms of ROS-mediated thrombopoiesis

While the majority of studies on ROS have traditionally focused on their deleterious biological effects, there is a growing body of evidence highlighting the importance of ROS as key mediators in various signaling pathways, particularly those related to hematopoiesis [55]. In the context of MK development, ROS function as second messengers in signal transduction, playing a crucial role in activating signaling pathways that are central to MK development and platelet production.

CD41 is a cell surface protein and a critical marker used to identify and characterize MKs. Research on thrombosis has demonstrated that $CD34^+$ peripheral blood cells produce more $CD41^+$ MKs under conditions with higher oxygen levels (20 % pO_2) than under conditions with lower oxygen levels (5 % pO_2) [56].

Both NF-E2 and Nrf2 are members of the Cap 'N' Collar (CNC) transcription factor family, which shares analogous DNA binding specificities. However, they play distinct roles in various cellular processes. NF-E2 serves as a critical regulator of MK maturation and platelet production. On the other hand, Nrf2 is a protein involved in antioxidant defense and cellular protection, working to counteract oxidative stress and toxins within cells. During MK maturation, ROS levels and oxygen tension increase, whereas the expression of stress-responsive genes responsible for ROS elimination decreases. In this context, NF-E2 competes with Nrf2 for controlling the expression of cytoprotective genes. This competition ultimately favors the accumulation of ROS, which is conducive to MK maturation [57].

Dopamine, a catecholamine neurotransmitter, can stimulate platelet

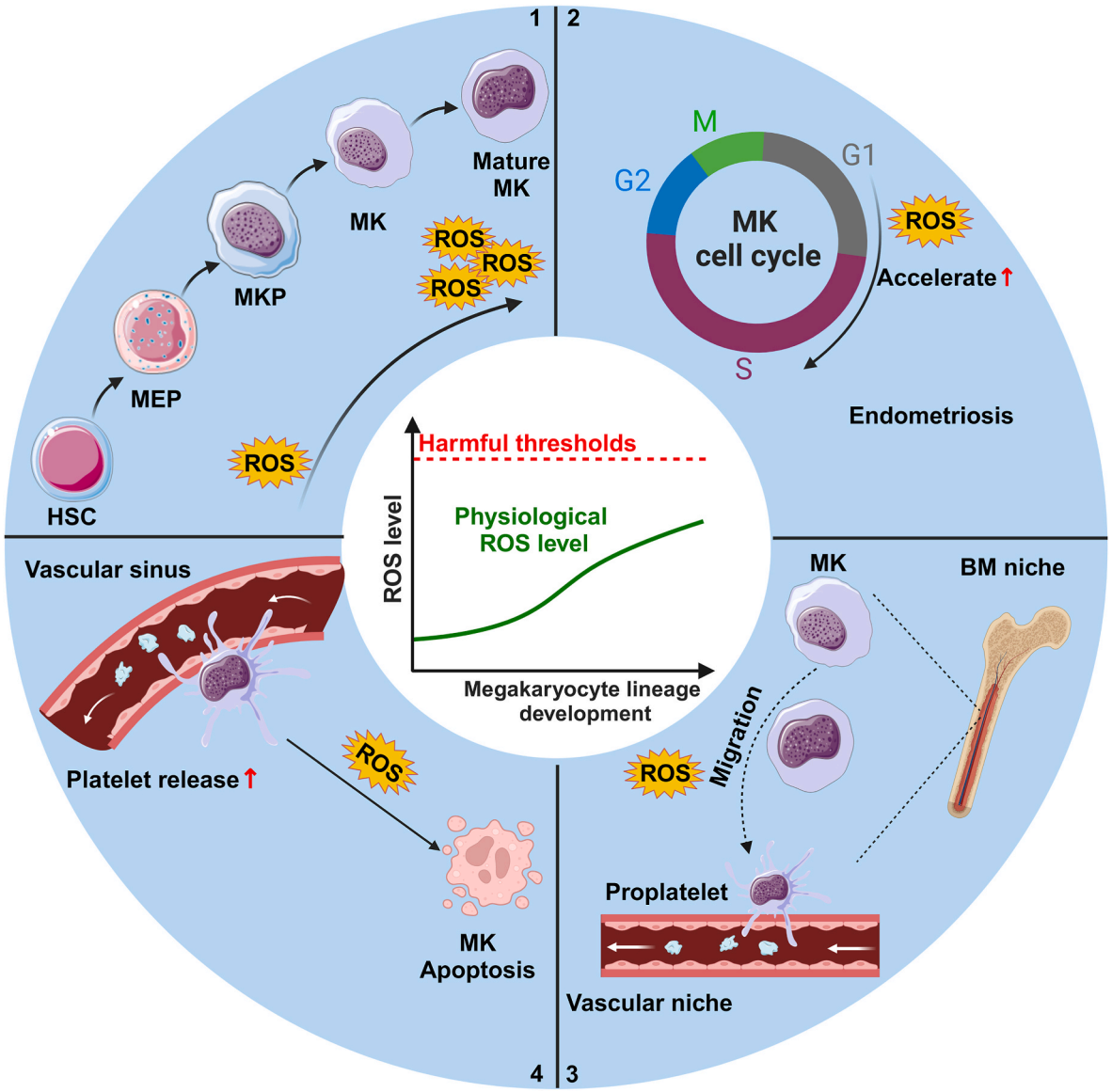

Fig. 4. Regulation of ROS during MK differentiation and platelet production. ROS play a crucial role in the process of thrombopoiesis. A carefully titrated level of ROS acts as a choreographer, guiding each step of the process. 1. Progressive differentiation of HSCs into mature MKs, accompanied by a gradual increase in ROS levels in the hematopoietic microenvironment; 2. ROS in the microenvironment accelerate mitotic G1 to S phase and polyploid formation in the MK nucleus and promote MK development to ensure that platelets have sufficient genetic material. 3. ROS induce MK migration from the bone marrow niche to the vascular niche and release platelets into the circulation in response to vascular shear. 4. Elevated ROS levels in the bone marrow microenvironment induce cytoskeletal changes in MKs, leading to the formation of proplatelets and promoting their migration toward the vascular endothelium, where they ultimately form platelet buds under vascular shear forces. The rise in ROS at this stage supports proplatelet formation, as well as MK apoptosis, ultimately facilitating platelet release. Notably, the physiological process of MK differentiation and platelet release is accompanied by elevated ROS levels. But this high ROS level remains physiological and does not reach the harmful threshold.

production independently of its receptor or transporter. Instead, it achieves this by triggering the activation of ROS-mediated oxidative stress-related signaling pathways, including the JNK/SAPK and p38MAPK pathways, as well as the caspase-3 pathway [58]. Photobiomodulation therapy (PBMT) is a noninvasive physical therapy that holds promise for alleviating chemotherapy-induced thrombocytopenia. PBMT achieves this by stimulating the production of TPO through the activation of the ROS-mediated Src/ERK/STAT3 signaling pathway, ultimately leading to an increase in platelet production [59]. ROS can also activate many other hematopoietic signaling molecules, such as Shc, STAT5 [60], SOS and downstream RAS, c-Kit [61], and PKC [62]. The JUN/FOS transcription factor family of p53, NF-κB and AP-1 can also respond to redox signal transduction [63].

A recent study highlighted the significant role of neutrophils in platelet production. Researchers have demonstrated that circulating neutrophils home back to the bone marrow and interact with perisinusoidal MK extensions, known as proplatelets, which are slender MK protrusions. Like MKs, neutrophils express NOX2 and have the ability to generate ROS [64]. These neutrophils expedite the shedding of PPLs through ROS-mediated mechanical signals within PPLs. This process is accompanied by the phosphorylation of ERK and MLC, which are both vital for MK maturation and PPL formation. Consequently, this mechanism leads to an increased platelet count [65] (Fig. 5). These findings collectively suggest that ROS likely play crucial roles in regulating various signaling pathways involved in MK differentiation and thrombopoiesis. However, the specific mechanism by which ROS regulate platelet production is still not fully understood.

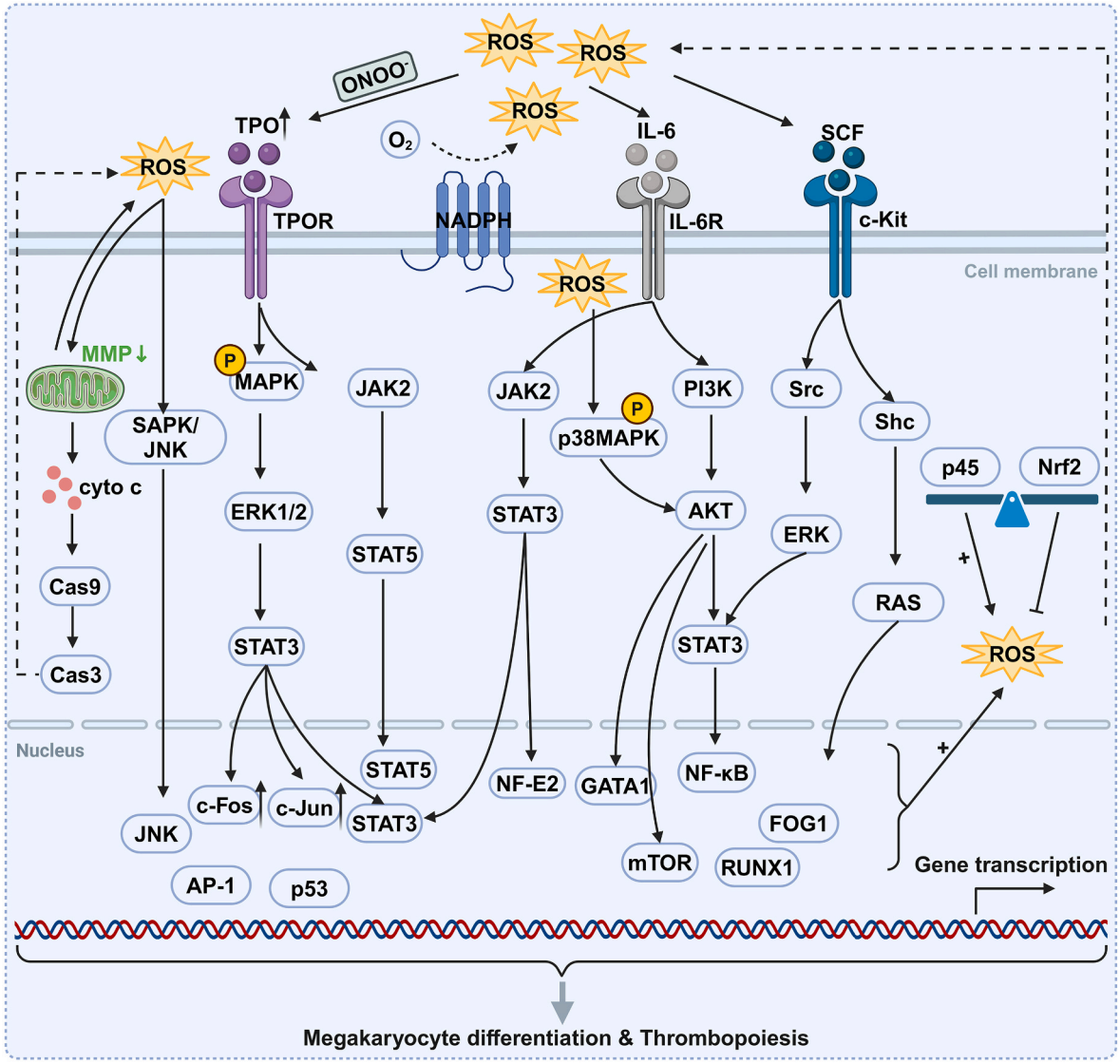

Fig. 5. Diagram of the mechanism of ROS-regulated thrombopoiesis. As crucial second messengers in a dynamic feedback loop, ROS not only activate key signaling pathways involved in megakaryocyte maturation and platelet production but are also regulated by multiple proteins and transcription factors. This interplay between ROS and signaling pathways orchestrates megakaryocyte function and ultimately governs platelet formation. However, the mechanism of platelet regulation by ROS is still far from perfect and will be an interesting area for future research. MMP, Mitochondrial membrane potential, ΔΨm.

7. Platelet adhesion, aggregation, activation and thrombosis

Hemostasis, the body's intricate clotting process, has crucial functions in maintaining vascular completeness and preventing excessive bleeding. When a vessel is injured, smooth muscle rapidly contracts, similar to a clamp, to minimize bleeding [66]. Simultaneously, the endothelial layer is disrupted, revealing collagen fibers. Like dancers, platelets take center stage, adhering to the exposed collagen and VWF through the specific receptors glycoprotein (GP)Ibα and GPVI of the GPIb-IX-V complex [67–69]. This initial adhesion sets the stage for further engagement. Subsequently, the platelets are activated, the internal Ca^{2+} concentration increases, and their morphology begins to change. They morph into spiky shapes, extending pseudopods such as tireless performers. These projections anchor platelets to the collagen fibers, further strengthening their grip [70]. After platelet activation, the integrin receptor αIIbβ3 on the platelet surface becomes activated and exposed, binding to fibrinogen in plasma. This interaction prompts the release of platelet granules, such as ADP and thromboxane A_2 (TXA_2), which further activate the platelets, induce vasoconstriction, and cause the aggregation of more platelets at the site of vascular injury, ultimately leading to the formation of a preliminary platelet plug called a thrombus [66,71]. The above process is called primary hemostasis. Then, the tissue factor (TF) at the site of injury is exposed and binds to coagulation factor VII (FVII) in the blood, activating it to FVIIa and forming the TF-FVIIa complex. This TF-VIIa complex further activates factor X (FX) to FXa, initiating the extrinsic coagulation pathway. Additionally, the intrinsic coagulation pathway is activated: first, FXII is activated to FXIIa, which subsequently activates FXI to FXIa, and finally, FXIa activates FIX to FIXa. Activated FIX combines with factor VIIIa to form a complex that further activates FX to FXa. Subsequently, FXa combines with factor Va to form the prothrombinase complex, catalyzing the conversion of prothrombin to thrombin. In summary, the coagulation cascade is initiated by both the extrinsic and intrinsic pathways and ultimately converges on the common coagulation pathway [72,73]. Subsequently, the generated thrombin promotes the conversion of fibrinogen to fibrin, forming an initial fibrin mesh and activating other coagulation factors (such as factors V, VIII, and XI). The release of thrombin and fibrin also facilitates the binding of activated platelets to fibrin via integrin receptors (such as αIIbβ3), further enhancing platelet aggregation. Additionally, factor XIII (fibrin cross-linking enzyme)

promotes the cross-linking of fibrin strands, thereby increasing the stability of the thrombus. Concurrently, the fibrin mesh interacts with aggregated platelets and red blood cells to create a stable thrombus that effectively prevents blood loss. This process is referred to as secondary hemostasis [74–77]. Once the wound heals, the body gracefully dismantles the temporary stage. Fibrinolytic enzymes, such as skilled stagehands, breakdown the fibrin mesh, dissolving the clot [78,79]. This process, known as fibrinolysis, ensures a smooth transition back to normal blood flow. In summary, hemostasis is a breath-takingly coordinated dance orchestrated by platelets and a supporting cast of proteins. This dynamic process ensures timely wound closure while maintaining a delicate balance of blood flow, safeguarding our health and well-being. After platelet activation, it is cleared by phagocytes to maintain platelet homeostasis.

8. Positive effects of ROS on platelet function

In recent years, a number of studies have revealed the crucial regulatory role of ROS, which act as second messengers in signal transduction, in platelet function [80]. NOX is a major contributor to ROS in the cell and has several isoforms. NOX is a major contributor to ROS in cells and has several isoforms, of which NOX1 and NOX2, the major NOX isoforms in platelets, are important sources of ROS in platelets and are thought to be responsible for platelet activation [69,81]. Both isoforms are inactive in resting platelets and are selectively activated by different platelet agonists, forming enzymatic complexes with several cytoplasmic proteins. This leads to the generation of ROS and regulates platelet function [23].

NOX1 is primarily activated by collagen stimulation, leading to the generation of ROS [22]. When collagen binds to GPVI on the surface of platelets, GPVI becomes activated. The activated GPVI promotes the phosphorylation of its intracellular tyrosine residues through Src family kinases (such as Src and Syk), further inducing the activation of the small G protein Rac. This, in turn, facilitates the binding of NOX1 to the cytoplasmic subunits p40phox, p47phox, and p67phox within platelets [82,83], forming a complete active enzyme complex [23]. Following the activation of the NOX1 enzyme complex, electrons are transferred from NADPH to molecular O_2, generating O_2^- and consequently leading to the production of ROS. The ROS generated by NOX1 not only enhances the binding of GPVI to collagen and induces the production of TXA_2 [22,84], but also further enhances platelet function by activating other signaling pathways, such as PI3K/AKT [85].

NOX2 primarily generates ROS in platelets through thrombin activation [22]. Thrombin activates the receptor by binding to the thrombin receptor PAR4 on the surface of platelets, which subsequently triggers the activation of G proteins (such as Gq and Gs). The activation of G proteins promotes the activation of Src kinases, leading to the tyrosine phosphorylation of downstream proteins such as Syk [69,83]. Similar to NOX1, the activation of Rac is a crucial step in the activation of NOX2 [86]. Activated Src kinases can further activate Rac, facilitating the translocation of the cytoplasmic auxiliary subunits p40phox, p47phox, and p67phox, which then associate with NOX2 to form a complete enzyme complex [23]. In the active NOX2 enzyme complex, NADPH acts as an electron donor, transferring electrons to molecular O_2 to generate ROS. The ROS produced by NOX2 not only activates the platelet integrin receptor αIIbβ3 and enhances its binding to fibrinogen but also induces the release of platelet granules and increases the expression of P-selectin, thereby promoting platelet adhesion and aggregation [87,88]. Additionally, NOX2-generated ROS can activate the JAK/STAT pathway, further enhancing platelet function [89]. Although NOX1 and NOX2 have different effects on platelet signal transduction and activation, their functions can be complementary, working together during the process of platelet activation. For instance, both are involved in collagen-induced thrombosis under arterial shear stress, but NOX2 plays a more prominent role in thrombus formation *in vivo* [84,90]. In addition, early studies have shown that rapid platelet activation results from acute inhibition of endogenous NO production [91], whereas ROS can react with platelet-derived NO to generate $ONOO^-$, another ROS species, thereby reducing NO bioavailability and partially enhancing platelet adhesion and aggregation [43].

Conversely, defects in platelet NOX1 and NOX2 lead to reduced ROS production and impaired platelet function [81,90]. NADPH oxidase inhibitors and superoxide scavengers hinder platelet aggregation via integrin αIIbβ3 activation by suppressing ROS production. ROS donors, such as 2,3-dimethoxy-1,4-naphthoquinone (DNMQ) [92], increase platelet activation, whereas ROS inhibitors, such as SOD and DPI, significantly impede collagen-induced platelet activation, aggregation, and thrombosis by reducing the release of platelet granules [93,94]. Another ROS inhibitor, NAC, has also been reported to inhibit platelet aggregation, activation, and plaque regression via ROS [89] scavenging (Fig. 6). Therefore, ROS make essential contributions to platelet function. In conclusion, these findings suggest that platelets are both a source and a target of ROS, which are involved in regulating various stages of thrombosis and hold promise as promising therapeutic approaches for diseases with platelet dysfunction.

9. Negative effects of ROS on platelet production

While moderate levels of ROS are vital for MK development and platelet production, excess ROS can impair platelet production. The human hematopoietic system is particularly sensitive to ionization radiation (IR) [95]. This brutal assault triggers a flood of ROS, leading to a cascade of damage: bone marrow dysfunction, MK apoptosis, and, ultimately, decreased platelet counts [96]. Conversely, the inhibition of ROS production with antioxidants can attenuate this damage [97]. In addition to causing immediate harm, chronic oxidative stress from high levels of ROS affect the self-renewal, proliferation and differentiation of HSCs and impair cell growth, resulting in a long shadow in terms of cell growth and development [98]. Thus, maintaining a delicate balance of ROS is crucial for healthy thrombopoiesis.

10. Negative effects of ROS on platelet function

The most relevant biological processes critical for regulating platelet function include the regulation of mitochondrial function, apoptosis, and oxidative stress. ROS can increase the exposure of phosphatidylserine (PS) on the surface of platelets and the formation of the mitochondrial permeability transition pore (mPTP) [22,99]. During platelet activation, the same is accompanied by increased platelet ROS generation and PS exposure [99]. Platelets contain a variety of anti-apoptotic or proapoptotic proteins, such as BCL-2, BAK and BAX. In general, when ROS mediates platelet activation, BAK and BAX are activated. These proteins form pores in the mitochondrial membrane, leading to the release of cytochrome *c* and subsequently activating downstream apoptotic signaling pathways, such as caspase-3. Activation of caspase-3 induces cytoskeletal remodeling and membrane structural changes, further promoting the translocation of platelet PS from the inner to the outer membrane, thereby forming a procoagulant platelet phenotype and enhancing its procoagulant activity [100,101]. The generation of thrombin in plasma depends on the exposure of platelet PS. The exposed PS not only continuously supports the generation of thrombin, but also attracts other activated platelets to aggregate, helping to form a stable blood clot at the site of injury and prevent blood loss [102]. However, the continuous stimulation of excessive ROS can lead to PS externalization, excessive platelet activation and excessive platelet apoptosis, which are important causes of excessive thrombosis and thromboembolic complications in oxidative stress-related diseases [6]. Furthermore, excessive platelet PS eversion provides binding sites for coagulation factors, allowing platelets to form a procoagulant phenotype, thereby promoting coagulation reactions. This process further enhances the activation and procoagulant function of platelets, increasing the risk of thrombosis [103]. In addition, hyperactivated

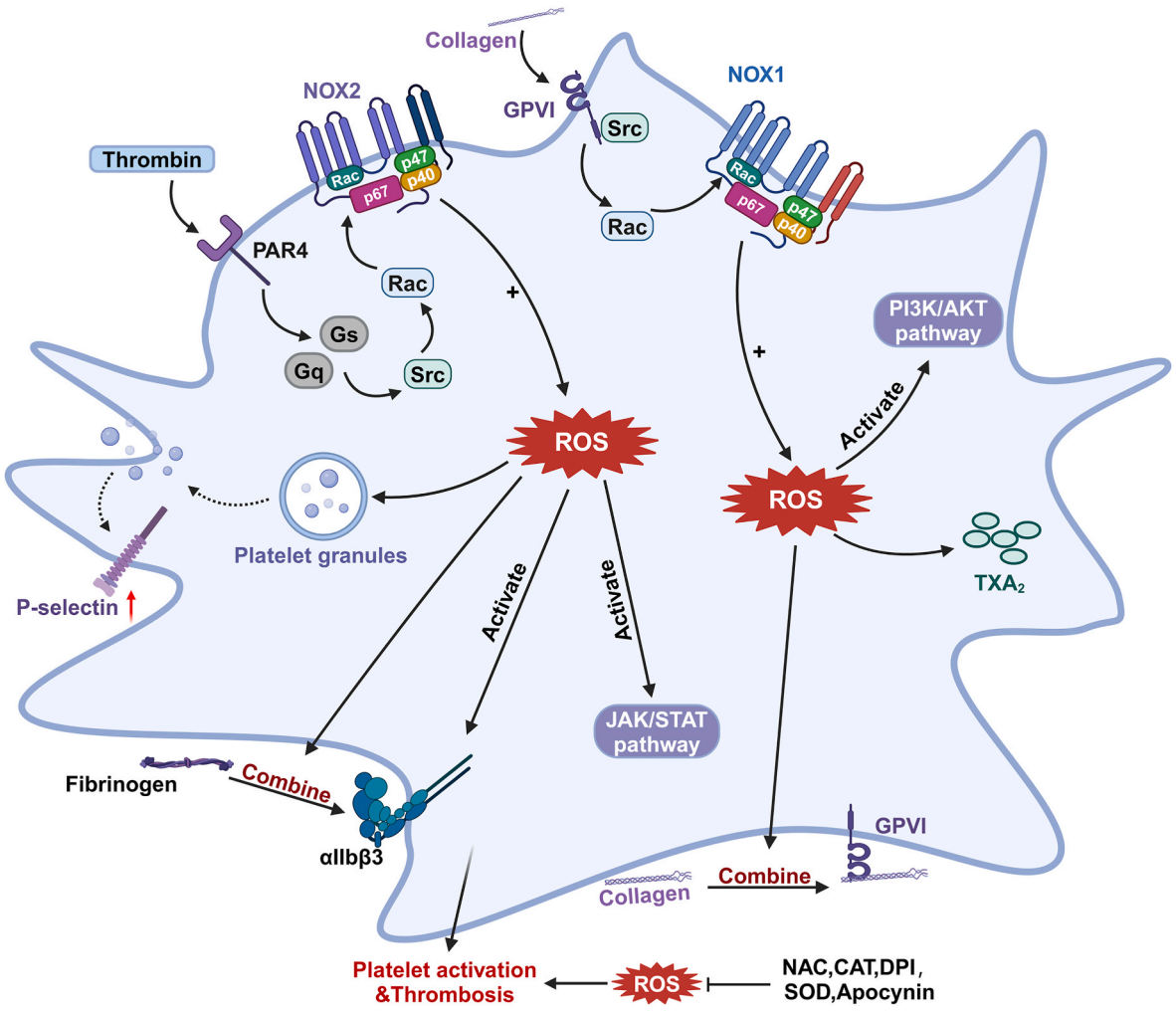

Fig. 6. ROS modulate platelet function. NOX1 and NOX2 are important sources of ROS in platelets and are considered to be responsible for platelet activation. Collagen and thrombin lead to the generation of platelet ROS through platelet NOX1 and NOX2, respectively. ROS produced by NOX1 not only enhance the binding of GPVI to collagen but also induce the generation of TXA2 and further augment platelet function by activating the PI3K/AKT pathway. Similarly, ROS generated by NOX2 activate platelet integrin αIIbβ3, strengthen its interaction with fibrinogen, induce the release of platelet granules, and upregulate P-selectin expression, thereby promoting platelet adhesion and aggregation. Additionally, NOX2-derived ROS activate the JAK/STAT pathway, further enhancing platelet functionality. ROS are important regulators of thrombus formation *in vivo* and can stimulate platelet activation. Reasonable ROS levels can promote various physiological processes of coagulation, such as mediating the binding of GPVI to collagen, promoting the release of platelet granules, activating integrin αIIbβ3, and increasing the expression of platelet P-selectin. Therefore, platelet activation and coagulation are accompanied by ROS production. Excessive ROS can lead to platelet overactivation, increasing the risk of thrombosis. Certain antioxidants, such as CAT, SOD, and NAC, can inhibit ROS-induced platelet activation, helping to prevent thrombosis resulting from ROS-mediated excessive platelet activation. The interaction between ROS and antioxidants maintains hemostatic homeostasis *in vivo*. DPI, diphenyleneiodonium chloride.

platelets display multiple apoptotic-like morphological changes, including exposure of PS, cell shrinkage, and microparticle (MP) formation. Apoptotic platelets shed MP carrying PS [104]. In addition, excessive ROS promotes platelet activation and aggregation by acting on platelet surface receptors, such as GPVI and αIIbβ3, and increases the production of TXA_2 in platelets and the release of platelet granules, further promoting platelet aggregation and thus increasing the risk of thrombosis [105]. In contrast, early studies demonstrated that platelet NOX2 deficiency and impaired ROS production resulted in reduced platelet activation [106]. Either too high or too low a level of ROS can disrupt the balance, leading to consequences such as hypofunction, increased activation, and even thrombosis. Therefore, the key to healthy platelet function is to maintain the balance of ROS levels.

11. Negative effects of ROS on platelet lifespan

Maintaining platelet count homeostasis demands a delicate balance not only between production and clearance but also between lifespan regulation [107]. Although historically restricted to nucleated cells, apoptosis has been recently demonstrated in platelets, which act as the primary physiological regulator of their lifespan [108]. During platelet apoptosis, survival signals mediated by Bcl-xL are inhibited, while the proapoptotic Bak/Bax complex is activated. This triggers a cascade of events within the mitochondria, culminating in inner membrane depolarization, cytochrome C release and subsequent activation of the caspase cascade in the cytoplasm [109]. This cascade, initiated by caspase-9 and culminating in effector caspases 3 and 7 [110], ultimately dismantles the cell, ensuring its controlled removal and maintaining platelet homeostasis crucial for hemostasis, coagulation and normal immune function [111]. Excessive apoptosis and clearance of platelets are also causes of thrombocytopenia [112]. In this way, it is clear that platelet clearance has an essential role in homeostasis. Apoptosis can be induced by a wide range of extrinsic and intrinsic signals, such as ROS. While low levels of ROS, such as H_2O_2, can support

cell survival, high doses trigger cell death processes such as apoptosis [113].

Given the absence of a nucleus, the lifespan of platelets is principally regulated by how well their mitochondria function. Notably, mitochondria are the major source of ROS in platelets, which can trigger apoptosis. Acting as the cell's powerhouses, mitochondria also hold the key to a different kind of energy: the controlled demolition of unwanted cells through apoptosis. This vital process is closely intertwined with the levels of ROS they generate. At low levels, ROS can act as signaling molecules, supporting cell survival. However, when the mitochondrial electron transport chain progresses, ROS levels increase, transforming these powerhouses into executioners [114]. Excessive ROS produced by mitochondria damage their own membranes, disrupting the energy-generating chain, leading to mitochondrial dysfunction and impaired ATP synthesis. The weakened membranes become leaky, allowing the release of cytochrome C, a key player in apoptosis. Once outside, cytochrome C binds apoptotic protease-activating factor-1 (Apaf-1) [113], a proapoptotic protein, triggering the activation of caspase-9. Caspase-9 then activates caspase-3 and caspase-7, causing the externalization of PS, ultimately leading to apoptosis [115–117]. This PS eversion is one of the characteristics of apoptotic cell phenotype. After PS eversion is exposed on the platelet surface, it will be recognized by macrophages, promoting the clearance of apoptotic platelets [118]. Notably, the mitochondrial-induced apoptosis process itself generates additional ROS in a caspase-dependent manner. Inhibiting caspase activation blocks ROS production. Upon activation, caspase-3 targets complex I and II of the mitochondrial electron transport chain, causing a sustained loss of ΔΨm and further ROS generation, which may intensify the apoptotic cascade. These newly produced ROS increase oxidative stress, potentially leading to further platelet apoptosis and accelerated clearance, thereby creating a vicious cycle that further reduces the number of platelets [119–122]. These findings suggest a potential regulatory capacity of ROS to modulate platelet apoptosis as well as lifespan. Zhang et al. demonstrated that cisplatin, a widely used antitumour drug, significantly elevated platelet ROS levels. This elevation was associated with upregulation and transposition of the apoptotic proteins Bax and Bak, downregulation of the antiapoptotic proteins Bcl-2 and Bcl-xL, depolarization of the mitochondrial membrane potential, liberation of cytochrome C, and triggering of caspase-3, as well as ERK and PS exposure. Importantly, ROS scavenging significantly inhibited cisplatin-induced platelet apoptosis, highlighting the causal role of ROS in this process [123]. ROS can increase platelet Bax levels and the phosphorylation of MAPKs (ERK, p38 and JNK) as well as decrease Bcl-2 levels. This cascade reaction leads to a decrease in the mitochondrial membrane potential, which ultimately leads to platelet apoptosis and a shortened lifespan.

In addition, cell membranes rich in polyunsaturated fatty acids (PUFAs) and organelle membranes are especially susceptible to ROS-mediated damage [124]. Similarly, in platelets, excessive ROS are inducers of oxidative stress and lipid peroxidation, leading to several detrimental consequences: decreased antioxidant capacity, structural alterations in platelet membranes, reduced energy availability, impaired metabolic activity, increased lactate dehydrogenase release and, ultimately, cell death [125]. Notably, the mitochondrial-induced apoptosis process generates additional ROS, which may exacerbate the apoptotic cascade. This occurs because, following the permeabilization of the outer mitochondrial membrane and the activation of caspase-3, caspase-3 targets complexes I and II of the electron transport chain, leading to a sustained loss of ΔΨm and the production of more ROS [126]. Fortunately, reactive oxygen scavengers such as NAC can mitigate this oxidative stress, protecting platelets and prolonging their lifespan [127, 128]. Consequently, excess ROS lead to oxidative stress, which activates the apoptotic pathway in platelets, thereby shortening the lifespan of platelets and ultimately leading to thrombocytopenia and bleeding (Fig. 7).

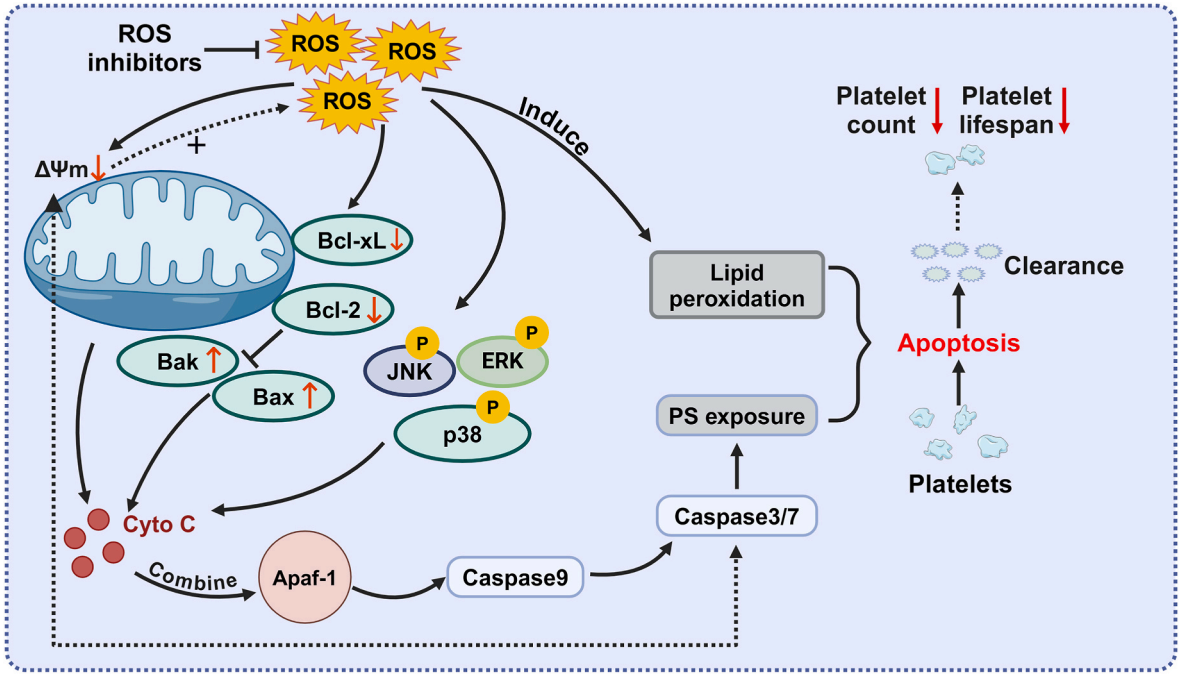

Fig. 7. Excessive levels of ROS impair platelet lifespan. Excess ROS disrupt the redox balance of platelets, increase the expression of the apoptotic proteins Bax and Bak, decrease the mitochondrial membrane potential, lead to the phosphorylation of p38MAPK, ERK and JNK, and facilitate the liberation of cytochrome *c* and its binding to Apaf-1, thereby activating mitochondrial caspase pathway-induced apoptosis. Excess ROS also cause lipid peroxidation in platelets. All of these factors lead to apoptosis, increase platelet clearance, and shorten the platelet lifespan, thus severely reducing the number of platelets. ROS-induced mitochondrial apoptosis in platelets can generate additional ROS through the activation of caspase-3, leading to a sustained loss of ΔΨm thereby creating a vicious cycle. Notably, antioxidants such as NAC can significantly attenuate platelet apoptosis. Cyto c, Cytochrome *c*; ΔΨm, mitochondrial membrane potential.

12. ROS generation inducers and antioxidants

Recognizing the critical role of ROS in the hematopoietic system, particularly in MK differentiation and platelet production, it has been identified as a significant therapeutic target for regulating platelet production and function. Here, we present a list of factors that have been documented to either induce ROS production or confer resistance to ROS. These factors help regulate the ROS levels within the body, thereby influencing platelet production and activation. They are categorized into chemical, physical, drug, receptor, cytokine and so on. This compilation serves as a valuable resource for exploring potential treatments for diverse conditions linked to ROS imbalance, including thrombocytopenia or thrombotic diseases (Table 1).

13. Conclusions, challenges and prospects

ROS, once relegated to byproducts of cellular respiration, have emerged as fascinatingly versatile signaling molecules in the hematopoietic system. However, the delicate balance between ROS and platelet homeostasis requires precise control within a narrow window of physiological relevance. This review delves into this intricate dance, highlighting the dual nature of ROS and the critical need for the regulation of platelet homeostasis. Platelet homeostasis is mainly determined by platelet number and function. This review cites a large amount of evidence showing that different levels of ROS have different effects on platelet number and function. Excessive ROS can activate excessive platelet aggregation and lead to excessive platelet apoptosis, thereby causing thrombosis or thrombocytopenia. Conversely, insufficient ROS levels hinder platelet production and suppress platelet activation, reduce platelet adhesion, impair survival, and thereby elevate bleeding risk. These findings underscore the dual role of ROS in platelet homeostasis. Only a balanced ROS level supports optimal regulation of platelet production, function, and lifespan. Therefore, maintaining a fine-tuned balance of ROS within the hematopoietic microenvironment is paramount. However, this pursuit necessitates addressing several key scientific challenges.

(1) Defining the "Goldilocks zone" of ROS: The precise ROS levels that promote healthy MK differentiation and platelet production without transitioning into pathological effects remain elusive. Extensive experimentation is crucial to establish this critical boundary.
(2) Untangling the signaling network: The human body employs numerous pathways for MK differentiation and platelet production, involving various factors such as cytokines. Investigating the complex interplay between ROS and these factors and how ROS modulate their activation to ensure stability and safety is crucial.
(3) Pinpointing the sources of ROS: While sources such as mitochondria and NADPH are recognized, the specific factors directly impacting platelets remain unclear. Additionally, deciphering the role of ROS generated by activated platelets themselves is crucial for obtaining a comprehensive understanding.
(4) Navigating the delicate balance of platelet life and death: Different ROS levels can both drive platelet production and trigger apoptosis, shortening the lifespan of platelets. Unveiling the precise mechanisms underlying this effect will allow for targeted interventions to maintain healthy equilibrium.
(5) Unraveling the ROS-mediated signaling pathways: While we know that ROS activate signaling pathways that promote platelet production, the precise nature of this activation remains unclear. Are these pathways directly activated or mediated through intermediate molecules or other pathways? Further investigation is needed. Treatment strategies for platelet disorders could be further improved by focusing on these issues.

Table 1
ROS modulators and their pharmacological effects.

ROS modulators	Cells	Disease	Pharmacological effect
Lithium chloride	Dami	–	Activating the wnt signaling pathway and increasing mitochondrial ROS biogenesis, which induces MK maturation and thrombopoiesis [129].
Biliverdin IXβ reductase B	$CD34^+$	Thrombocytopenia	Increasing ROS levels can promote MK differentiation and thrombopoiesis, which can be used as a new therapeutic target for thrombocytopenia [130].
PMA	K562	Dengue	Promoting the accumulation of ROS in K562 cells, inducing the differentiation of cells into mature MKs and the expression of platelet surface marker CD41/CD61 [51].
Justicia adhatoda	Dami	–	Enhancing the mitochondria-derived ROS generation and increasing in mitochondrial membrane permeability and promoting upregulation of RUNX1 as well as c-Mpl expression, thereby inducing megakaryocyte maturation [131].
Dopamine	$CD34^+$	–	Inducing ROS generation, then activating JNK/SAPK, p38 MAPK and caspase-3, ultimately inducing thrombopoiesis [58].
15d-PGJ2	Meg-01 and Dami	Thrombocytopenia	Enhancing cellular ROS production, inducing MK polyploidy formation, accelerating platelet production, and having the ability to treat radiation-induced thrombocytopenia [16].
PBMT	Meg-01	Thrombocytopenia	Inducing ROS production, which activate the Src/ERK pathway, then increasing hepatic TPO and ultimately promoting thrombopoiesis [59].
X-ray	K562	–	Increasing cellular ROS production through activation of the MAPK signaling pathway, leading to increased CD41 expression and megakaryocyte maturation [132]
Ionizing radiation	M07e	Thrombocytopenia	Stimulation of NADPH produces ROS and activates the JNK/p38 MAPK pathway, leading to megakaryocyte apoptosis [96].
IL-6	HAEC	COVID-19	IL-6 selectively activates NOX2 to accelerate ROS generation, exacerbate endothelial oxidative stress, and maintain endothelial dysfunction and vascular inflammation [133].
TLR2	Dami	–	Induction of ROS production, activation of wnt signaling cascade response, then promotion of CD41 expression and MK maturation [134].
Collagen/ Thrombin/ Thromboxane	Human platelet	–	They promote the generation of nitrogen oxides, leading to increased intracellular ROS,

(*continued on next page*)

Table 1 (*continued*)

ROS modulators	Cells	Disease	Pharmacological effect
			activation of platelets, and ultimately thrombosis [94].
LPS	Human platelet	–	Promoting platelet P-selectin expression and ROS generation by binding to platelet TLR4. Generated ROS enhance platelet aggregation by phosphorylating AKT, PKC and p38 [135].
Labile iron pool	NFS60	–	Stimulation of intracellular ROS production by bone marrow hematopoietic cells [60].
NAC	Human platelet	Platelet storage lesion (PSL)	Inhibiting platelet ROS production, thereby reducing ROS-mediated platelet aggregation, apoptosis and thrombosis [136,137].
DPI	Human platelet and Meg-01	–	Inhibiting ROS production in human platelets and Meg-01 cells, and inhibiting platelet function [138].
SOD	HEK293T	–	Catalyzing superoxide into oxygen and hydrogen peroxide, which is then converted to oxygen and water by CAT, thereby balancing intracellular ROS levels and acting as a key antioxidant [139].
Apocynin	Human platelet		Inhibiting collagen-induced ROS and collagen-induced platelet aggregation, also inhibiting platelet ROS and TXA2 production [140].

Thrombosis is a complex and multicellular process. For example, the interaction between platelets and neutrophils also plays a key role in the coagulation function of platelets. Neutrophils are a potential link between hemostasis and innate immunity due to the prothrombotic potential of NETs. The discharge of depolymerized DNA strands from neutrophils can trigger coagulation through platelet and prothrombin activation and then further promote thrombosis [141]. In addition, the interaction between neutrophils and activated platelets can accelerate the release of NETs [142,143]. The decrease in platelet–neutrophil interactions and NET release in GPIbα-deficient mice also indicates that platelets contribute to neutrophil recruitment and NET release. ROS play important roles in the formation of neutrophil NETs. On the one hand, the NADPH oxidase-dependent pathway is involved in the formation of NETs through ROS production [144], and ROS also induce platelet activation. On the other hand, glucose oxidase (GO) further stimulates DPI-treated neutrophils to produce exogenous ROS and induces NET formation [145]. These results further elucidate the mechanism by which ROS regulate platelet function.

In addition, when platelets are activated, many microvesicles derived from the plasma membrane (accounting for 70–90 % of the plasma membrane), also known as procoagulant platelet microvesicles (PMVs) [146], are membrane-encapsulated extracellular vesicles (EVs) released by platelets after activation [17], which play a key role in platelet activation and thrombosis [147]. Oxidative stress-mediated signal transduction is a key pathway for platelet activation because ROS can induce the formation of PMVs. Specifically, by inducing the binding of metabolically active extracellular mitochondria (exMTs) to platelets through phospholipid-CD36 interactions, exMT produces ROS during this process. This direct exMT–platelet interaction may increase ROS activity in exMT-bound cells, and ROS act as intermediate media to activate platelets and accelerate PMV formation. The physical contact between exMT and platelets seems to be necessary for ROS-induced platelet secretion, as ROS affect mainly exMT-bound platelets [148]. In addition, the formation of PMVs can not only activate platelets but also induce oxidative stress. When these PMVs are released from activated platelets, they also carry key enzymes involved in ROS production [149] and can also induce oxidative stress through the JNK and NF-κB pathways [150]. PMVs further enhance platelet activation by triggering intracellular oxidative stress-mediated ROS production.

Platelet fibrinogen receptor αIIbβ3 is the most highly expressed integrin on the platelet surface, and the reduction of disulfide bonds on platelets mediated by its surface-expressed oxidoreductase protein disulfide isomerase (PDI) is importantly related to its function. It was demonstrated that the interaction of αIIbβ3 with fibrinogen leads to sustained binding of fibrinogen to αIIbβ3 and increased expression of platelet P-selectin through disulfide isomerization and disulfide bond exchange mediated by reduction of PDI, ultimately leading to platelet aggregation. In contrast, inhibition of PDI and disulfide bond exchange impedes platelet aggregation [151]. Other studies have shown that activation of the protein tissue factor (TF), a key step in the blood clotting process, leads to the formation of fibrin. By reducing the free sulfhydryl groups of TF, PDI activates TF through a specific type of disulfide bond exchange that allows PDI to induce the formation of intramolecular disulfide bonds in TF, which promotes fibrin formation and ultimately initiates blood clotting. At the same time, a fraction of the extracellular cysteine 209 of TF was oxidized, and oxidation of cysteine increased the procoagulant function of TF. These results provide further evidence for the regulation of thrombosis by redox processes [152].

VWF and coagulation factor VIII (FVIII) circulate in the plasma as complexes (VWF multimers). The two major receptors on the platelet surface, GPIbα and αIIbβ3, play an important role in the hemostatic system by interacting with VWF multimers to mediate platelet deposition into damaged vessel walls [153]. Only large VWF multimers were effective in promoting platelet adhesion to the site of vascular injury. In contrast, a decrease in mean VWF multimer size correlated with bleeding propensity, suggesting that regulation of VWF multimer size is critical in arterial thrombosis. Studies have shown that platelet disulfide bond reductase activity plays an important role in the regulation of platelet VWF multimer size [154]. Xie et al. found that redox regulation is involved in the control of VWF multimer size. Incubation of VWF with the protein reductants, protein disulfide isomerase and thioredoxin, resulted in formation of new thiols in VWF and reduction in the average size of VWF multimers [155]. Redox small molecules may also be involved in controlling the size of VWF multimers, such as the GSH-GSSH system associated with endothelial cells. Li et al. found that GSH can reduce VWF multimers to a smaller form [156]. These findings suggest that redox may have an effect on VWF multimer-mediated thrombosis. However, the redox-regulated processes of VWF multimers undoubtedly require further investigation.

Platelet disorders refer to diseases caused by abnormalities in platelet number and function. We propose that targeted regulation of ROS levels could be a therapeutic strategy for treating platelet disorders, such as thrombocytopenia induced by radiotherapy or chemotherapy, and thrombotic diseases. Excessive ROS are generated during cancer radiotherapy and chemotherapy, which induce thrombocytopenia by damaging mitochondria and activating apoptotic signaling pathways. Additionally, in the context of atherosclerosis, hypertension, and diabetes, ROS can accelerate platelet activation and thrombosis, leading to arterial thrombosis and thereby increasing the risk of cardiovascular events, such as myocardial infarction and stroke. Strategies aimed at either reducing or increasing ROS levels, depending on the pathological context, offer promising avenues for intervention. For example, targeting the p47phox subunit of NOX, a key ROS-generating enzyme, represents a potentially safer therapeutic approach than targeting other NOX subunits. Furthermore, platelet homeostasis can be more precisely regulated by designing and developing drugs or other therapeutic tools that target the regulation of ROS levels and spatiotemporal distribution. However, these therapeutic strategies require further exploration and validation in clinical trials before they can be translated into clinical

applications.

In summary, the multifaceted role of ROS in the hematopoietic system, particularly their impact on platelet production and function, reveals a fascinating story of dynamic regulation and delicate balance. While significant progress has been made, unanswered questions remain. Addressing these questions through future research will unlock the full potential of ROS-based therapies for treating platelet-related diseases and pave the way for novel therapeutic strategies. These challenges not only enhance the study of ROS but also offer promising strategies for regulating platelet homeostasis. This review aims to contribute to this ongoing dialog and inspire further exploration of this exciting field.

CRediT authorship contribution statement

Rui Liao: Writing – original draft, Investigation, Conceptualization. **Long Wang:** Writing – original draft, Investigation, Conceptualization. **Jing Zeng:** Writing – original draft, Investigation, Conceptualization. **Xiaoqin Tang:** Software, Methodology, Investigation. **Miao Huang:** Software, Methodology, Investigation. **Fahsai Kantawong:** Validation, Software, Investigation. **Qianqian Huang:** Validation, Methodology. **Qibing Mei:** Validation, Supervision, Resources. **Feihong Huang:** Validation, Investigation. **Yan Yang:** Supervision, Resources. **Bin Liao:** Writing – review & editing, Validation, Supervision. **Anguo Wu:** Writing – review & editing, Supervision, Conceptualization. **Jianming Wu:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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