Received: 23 October 2024 | Accepted: 24 October 2024

DOI: 10.1002/jha2.1051

eJHaem

British Society for Haematology

REVIEW

New insights into the mechanisms of red blood cell enucleation: From basics to clinical applications

Qianli Zhuo[1,2,3] | Zhaojun Zhang[1,2,3,4] | Xiangdong Fang[1,2,3,4]

[1]China National Center for Bioinformation, Beijing, China

[2]Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, China

[3]University of Chinese Academy of Sciences, Beijing, China

[4]Beijing Key Laboratory of Genome and Precision Medicine Technologies, Beijing, China

Correspondence
Zhaojun Zhang, and Xiangdong Fang, China National Center for Bioinformation, Beijing, 100101, China.
Email: zhangzhaojun@big.ac.cn and fangxd@big.ac.cn

Funding information
National Key Research and Development Program of China, Grant/Award Number: 2022YFC2406803; National Natural Science Foundation of China, Grant/Award Numbers: 82070114, 82270126

Abstract

Background: Red blood cell (RBC) enucleation is a crucial step in the process of erythropoiesis. By removing the nucleus, RBCs gain greater flexibility, enabling them to traverse narrow capillaries with ease, thereby enhancing the efficiency of oxygen and carbon dioxide transport. This transformation underscores the intricate balance between cellular structure and function essential for maintaining homeostasis.

Topic: This review delves into the multifaceted enucleation process, outlining its complex steps that encompass protein sorting, vesicle trafficking, cytoskeletal remodeling, and apoptosis regulation, while also exploring the potential of enhancing the enucleation rate of RBCs in vitro. We emphasize the intricate regulation of this process, which is orchestrated by multiple factors. This includes transcription factors that meticulously guide protein synthesis and sorting through the modulation of gene expression, as well as non-coding RNAs that play a pivotal role in post-transcriptional regulation during various stages of RBC enucleation. Additionally, macrophages participate in the enucleation process by engulfing and clearing the extruded nuclei, further ensuring the proper development of RBCs. Although many studies have deeply explored the molecular mechanisms of enucleation, the roles of apoptosis and anti-apoptotic processes in RBC enucleation remain incompletely understood.

Implication: In this review, we aim to comprehensively summarize the RBC enucleation process and explore the progress made in ex vivo RBC generation. In the future, a deeper understanding of the enucleation process could provide significant benefits to patients suffering from anemia and other related conditions.

KEYWORDS
apoptosis, biological fundamentals, enucleation, erythropoiesis, methodology, RBCs

1 | INTRODUCTION

Red blood cell (RBC), as the carriers of oxygen and carbon dioxide, is the key functional component of the hematologic system for survival. The capabilities of RBCs for the transportation of gaseous molecules depend on the functional integrity of hemoglobin in RBCs. [1]. However, unlike other somatic cells and hemocytes, RBCs characterize a unique morphological signature: they are anucleate [2, 3]. Unlike other

somatic cells, RBCs are anucleate, a feature that increases their capacity for hemoglobin storage and oxygen transport [4]. This anucleate characteristic also renders RBCs more flexible and deformable features, allowing them to easily traverse through tiny capillaries [5]. While the absence of a nucleus limits the lifespan and repair capabilities of RBCs, this specialized structural design is crucial for the primary function of RBCs.

In the evolutionary process of vertebrates, RBC enucleation is a relatively late-appearing phenomenon, primarily found in mammals. Other vertebrates, such as fish, reptiles, amphibians, and birds, retain the nucleus in their RBCs, which remains visible in mature RBCs. This difference reflects the diverse adaptive strategies that different organisms have developed to meet their oxygen transport needs during evolution. While RBC enucleation in mammals increases hemoglobin capacity and enhances oxygen transport efficiency, other animals, such as birds, can still meet their high oxygen demands during flight, despite retaining the nucleus in their RBCs. This demonstrates that distinct species have evolved different mechanisms to adapt to their physiological needs. Therefore, understanding the genesis and maturation of RBCs is crucial for comprehending their function. Erythropoiesis, the process of RBC formation, is a complex biological event, involving key stages such as chromatin condensation and enucleation. This process is regulated by signaling pathways activated by erythropoietin (EPO), which promotes cellular differentiation, and proliferation, and exerts anti-apoptotic effects [6, 7]. Nevertheless, the specific mechanism of erythroid chromatin condensation remains unclear, recent studies suggested that various modifications of histone tails contribute to this process [8]. Knockdown of histone deacetylases, such as HDAC2, impacts chromatin condensation and the subsequent enucleation process. The RBC enucleation was also identified, as the most crucial phase for RBCs generation [9, 10]. This process is recognized not just as a cellular activity, this process is regulated by the cooperation of genetic, epigenetic, and metabolic actions. Previous evidence from the studies of molecular biology and genetics provided valuable insights into the complex mechanisms behind RBC enucleation [11, 12], and offered valuable insights into the normal RBC enucleation process [13]. However, as new mechanisms of RBC enucleation and their significance for RBC cloning in vitro have been discovered continuously, a systematic and comprehensive review is essential for our deeper understanding of the regulatory mechanisms of RBC enucleation, involving epigenetic regulation, transcription factors, cytoskeletal dynamics, involving the process of apoptosis and anti-apoptosis, and cell autophagy in the (the mechanisms of RBC enucleation were depicted in Figure 1).

2 | CHROMATIN CONDENSATION IN RBC ERYTHROPOIESIS

Chromatin condensation is a critical step in the process of erythrocyte enucleation. During this process, the chromatin in precursor erythroid cells undergoes significant condensation, preparing for the physical removal of the nucleus. This extensive chromatin condensation leads to a reduction in nuclear size, facilitating the separation between the nucleus and cytoplasm. Subsequently, through a series of complex biological processes, the nucleus is completely expelled from the cell, resulting in the formation of enucleated mature RBCs. Wang et al.'s research demonstrated that during the maturation process of embryonic stem cells (ESCs), chromatin undergoes a series of dynamic changes, with the most notable being chromatin condensation and nuclear shrinkage after the formation of precursor erythroid cells. At this stage, the nuclear lamina also undergoes reorganization and partial degradation, providing sufficient space for the shrinkage and eventual extrusion of the nucleus [14]. Epigenetic modifications play a crucial role in chromatin condensation, promoting the transition of chromatin from a loose to a compact state through DNA methylation, histone acetylation, and chromatin remodeling. This transition is essential for erythrocyte enucleation. Studies have found that high expression of c-Myc protein prevents the deacetylation of specific lysine residues in histones H3 and H4. Elevated Myc levels also promote the expression of histone acetyltransferase Gcn5, inhibiting chromatin condensation [15]. Rossmann et al. showed that the chromatin factor TIF1γ regulates mitochondrial genes, linking epigenetic regulation during chromatin condensation to cellular metabolism. Their study found that TIF1γ directly controls the expression of coenzyme Q (CoQ) synthesis genes. Loss of TIF1γ leads to decreased CoQ levels, resulting in an increased succinate/α-ketoglutarate ratio, which triggers increased histone methylation and abnormal chromatin condensation [16]. Further research by Bhoopalan et al. revealed that EPO plays a key role in chromatin condensation by regulating the maturation of precursor erythroid cells and epigenetic modifications, highlighting the interplay between hormonal regulation and epigenetic control in erythropoiesis [17]. Semenza and colleagues discussed erythropoiesis regulation from the perspective of the hypoxia-inducible factor pathway, providing deeper insights into how environmental factors like hypoxia influence epigenetic regulators [18]. Under hypoxic conditions, HIF-2α promotes the production of EPO by upregulating the expression of the histone H3K9 demethylase JMJD1A, thereby promoting chromatin condensation [19]. In addition, extensive studies have revealed that histone modifications are a key target of epigenetic regulation. Caspase-3 (Casp3) was initially identified as a crucial regulator in this process. Casp3 is critical for chromatin condensation and is also involved in erythrocyte enucleation in mice [20]. Specifically, Casp3 regulates the formation of nuclear openings and histone release. Its inhibition impairs nuclear condensation, affecting enucleation. Similarly, the caspase-independent protein Wdr26 controls the formation of nuclear openings during terminal erythrocyte formation and regulates the ubiquitination and degradation of nuclear proteins. Its absence results in failed nuclear condensation [21]. During this process, many nuclear proteins, including histones, are exported to the cytoplasm, a key step in terminal erythroid maturation. In the final stages of terminal erythroid maturation, chromatin condensation mimics apoptosis, particularly through the formation of condensed chromatin and pyknotic nuclei. The non-apoptotic functions of caspases are closely related to erythrocyte maturation and enucleation [22]. Moreover, chromatin condensation involves the deacetylation of histones by HDACs, which

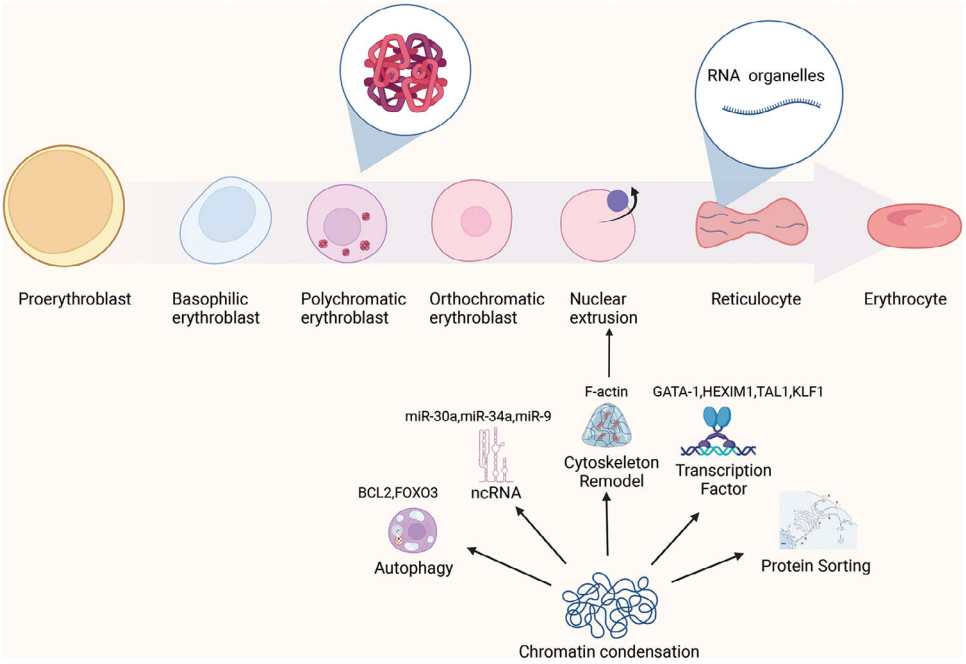

FIGURE 1 This diagram elaborates on the intricate developmental trajectory of proerythroblast differentiating into a mature erythrocyte. It meticulously depicts the significant morphological changes where the Proerythroblast gradually transforms into a Polychromatic erythroblast, initiating hemoglobin synthesis at this juncture, a pivotal step for the subsequent oxygen transport function of erythrocytes. As the developmental progression deepens, erythrocytes undergo a hallmark event-enucleation, the expulsion of the cell nucleus. This process necessitates the highly condensed state of chromatin and involves intricate molecular mechanisms, including precise protein sorting, the participation of crucial transcription factors such as gata1, hexim1, tal1, and klf1, dynamic remodeling of the F-actin cytoskeleton, regulation by non-coding RNAs (including miR-30a, miR-34a, miR-9, etc.), and intricate modulation of cellular autophagy. These molecular mechanisms work in concert, guiding the precursor erythroid cells through the transition from nucleated to anucleated erythrocytes, ultimately shaping the mature, anucleated erythrocytes with their characteristic biconcave discoid morphology. These erythrocytes are then released into the circulatory system to perform their core physiological functionâ€"oxygen transport throughout the body. The visual representation in this diagram profoundly reveals the delicate balance and interdependence between gene expression patterns, protein synthesis activities, and cellular structural reorganization, which are vital for the successful generation and maintenance of functional erythrocytes.

is crucial for erythroid maturation and enucleation [15]. HDAC1, 2, 3, and 5 are highly expressed in mouse erythrocytes. During chromatin condensation, HDAC1 and HDAC3 levels decrease, while HDAC2 and HDAC5 levels increase, accompanied by a reduction in H4K16ac levels [23, 24]. H4K16ac is a substrate of the deacetylase SIRT1, which regulates overall chromatin structure and is essential for terminal erythroid chromatin condensation. Although the specific mechanism by which HDAC-mediated chromatin condensation affects enucleation remains unclear, HDACs can directly or indirectly activate the Rac GTPase-mDia2 pathway to promote enucleation [25]. In addition, HDAC6 is essential for the formation of the contractile actin ring and enucleation, and mDia2 regulates the acetylation and deacetylation of lysine 970 during erythropoiesis to control chromatin condensation [26].

Chromatin condensation is a complex molecular event sequence. Caspase-3, Wdr26, Xpo7, and HDAC family proteins collectively participate in this process by regulating the export of nuclear proteins, histone modifications, and cytoskeletal reorganization. A deeper understanding of these molecular mechanisms is essential for elucidating the chromatin condensation process preceding erythrocyte enucleation.

3 | THE PROCESS OF RBC ENUCLEATION

3.1 | Protein sorting during the enucleation process

Protein sorting serves as a critical regulatory mechanism governing the enucleation process of erythrocytes. Protein sorting involves either the selective sorting of specific proteins or the non-specific redistribution of proteins, ultimately allocating them to the nucleus-containing cell (pyrenocyte) or the reticulocyte membrane. Cytoskeletal proteins, such as spectrin and ankyrin, which are crucial for erythrocyte function, are retained in newly formed reticulocytes [27]. Moreover, integral membrane proteins associated with the cytoskeleton, such as glycophorin A (GPA), RhAG, and glycophorin C (GPC), are predominantly located on the reticulocyte membrane. Although the mechanisms underlying the selective retention or exclusion of proteins in newly formed reticulocytes are not yet fully understood, they play a pivotal role in the erythrocyte enucleation process [27]. During erythrocyte enucleation, the cytoskeleton undergoes reorganization. This process facilitates the formation of large protein membrane networks that

regulate cell morphology by modulating protein-cytoskeleton interactions or ion transport, thereby maintaining the structural integrity of erythrocytes in circulation. These networks can be broadly categorized into ankyrin and junctional complexes [28]. Additionally, the proteins within these networks aid in the retention of reticulocytes during enucleation and vice versa; their absence can lead to disease. For instance, in 4.1-deficient erythrocytes, GPC is mislocalized to pyrenocytes, whereas it is typically sorted into reticulocytes. Similarly, in ankyrin-deficient erythrocytes, GPA and Rh-related antigens are incorrectly directed to pyrenocytes [29]. Therefore, understanding the mechanisms of protein sorting could potentially elucidate the entire process of erythrocyte enucleation in the future.

3.2 | Transcription factor regulation of the enucleation process

Transcription factors play a critical regulatory role in the enucleation process of erythrocytes, driving this process through their unique functions. During erythrocyte enucleation, the activity of specific transcription factors such as GATA-1, hexamethylene bisacetamide-inducible 1 (HEXIM1), TAL1, KLF1, and FoxO3 is upregulated. These transcription factors work synergistically to regulate the expression of erythroid-specific genes, thereby controlling the production of hemoglobin and other proteins closely related to erythrocyte function. In this way, they promote enucleation, ensuring the generation of fully functional, mature, anucleate erythrocytes. Early studies focused on the dynamic changes in DNA methylation at the β-globin locus during the transition from fetal to adult hemoglobin, revealing the dynamic nature of transcription factor-driven epigenetic regulation in the enucleation process [30, 31]. Lv et al. found that the transcription factor HEXIM1 regulates gene expression and function during erythrocyte enucleation by enhancing RNA polymerase II (RNAPII) activity. The interaction between HEXIM1 and GATA1 plays a key role in regulating RNAPII pausing and activating or repressing gene expression, which is crucial for the enucleation process [32]. Moreover, transcription factors such as GATA1 and TAL1 are considered core regulators in the enucleation process, orchestrating the complex gene expression patterns required for erythroid maturation. GATA1 is often regarded as the primary regulator of erythrocyte enucleation, essential for normal erythroid development. It controls the gene network that drives erythroid differentiation and promotes enucleation. TAL1 works synergistically with GATA1 to regulate erythroid differentiation [33]. Extensive research has confirmed TAL1's role in erythrocyte enucleation, demonstrating its critical function in erythroid maturation by influencing the expression of related genes [34]. Recently, Ohneda and colleagues highlighted the central role of GATA-1 and GATA-2 in gene regulation during the erythrocyte enucleation cascade [35]. They pointed out that GATA-2 is essential for maintaining and proliferating immature hematopoietic progenitor cells, while GATA-1 is crucial for erythrocyte survival and terminal differentiation. Li et al. discovered that transcription factor GATA1 is involved in maintaining the stability of topologically associating domains during erythrocyte enucleation. GATA1 regulates enucleation by preserving the integrity of chromatin domains during terminal erythroid differentiation [36]. Phosphatidylinositol 3-kinase (PI3K) activation plays a key role in EPO-induced erythrocyte enucleation [37, 38]. In the fetal liver of mice, PI3K-mediated nuclear polarization is essential for erythrocyte enucleation. Typically, FoxO3 is phosphorylated and inactivated by PI3K and its downstream effector Akt. However, during erythrocyte enucleation, FoxO3 expression and nuclear accumulation increase. Transcriptomic analyses revealed differential regulation of FoxO3 target genes [39]. Some FoxO3 target genes, such as Cited2, were not inhibited by EPO-induced PI3K activation. These findings underscore the pivotal role of FoxO3 in erythrocyte enucleation.

Transcription factors are crucial in the erythrocyte enucleation process. A deeper understanding of these mechanisms is vital for elucidating the complexity of erythropoiesis, enhancing our understanding of related hematological disorders, and developing targeted therapeutic strategies.

3.3 | Remodeling of the cytoskeleton during the enucleation process

The cytoskeleton plays a central role in the process of erythrocyte enucleation, as supported by extensive research. The cytoskeleton not only provides structural support for cells but also directly participates in the key steps of erythroid cell maturation and enucleation. (1) Actin cytoskeleton reorganization: crucial for the physical separation of the cell nucleus from the cytoplasm, with the formation of the actin contractile ring being key to the formation of anucleated reticulocytes. The dynamic adjustment of the actin cytoskeleton is vital for ensuring the smooth progression of erythrocyte maturation [40]. (2) The role of microtubules: essential in positioning the cell nucleus and forming the cell membrane, with effective coordination with actin filaments being a prerequisite for the successful completion of erythrocyte enucleation [41, 42]. (3) Intermediate filaments and related proteins: play an important role in maintaining the stability and integrity of erythroid cells during enucleation, participating in the mechanical process of nuclear extrusion [43]. During the terminal differentiation and enucleation process of erythroid cells, the erythroid membrane cytoskeleton undergoes dynamic remodeling, with a crucial role played by actin filaments (F-actin). Linear and branched structures of F-actin are formed through the actions of formin proteins and the Arp2/3-Wasp complex. Specifically, high expression of the formin protein mDia2 in erythroid cells is essential for the formation of the actin ring and the enucleation process. Loss of mDia2 can lead to impaired contraction of the actin ring during enucleation and the presence of multi-lobed nuclei in erythrocytes. Additionally, the RhoA-GTPase and its effector molecule mDia2 play a regulatory role in terminal erythrocyte generation, and their deficiency can result in early embryonic lethality and failure of the cytoplasmic division [44]. Apart from the RhoA-mDia pathway, other actin-regulatory factors such as Pleckstrin-2 and Dematin also contribute to erythrocyte enucleation, and their absence or knockout can disrupt the actin cytoskeleton and impede the enucleation process [45]. Fraser et al. have investigated the role of heme oxygenase-1 in erythrocyte production and turnover, emphasizing the importance of

cytoskeletal interactions in erythroid island formation and RBC lifespan [46]. The regulation of the cytoskeleton is influenced by various factors, including variations in key regulatory factors like KLF1, the activity of heme oxygenase-1, and intracellular oxygen sensing mechanisms [47]. These factors collectively regulate the dynamic changes in the cytoskeleton, affecting the processes of nuclear extrusion and erythrocyte maturation [48]. Therefore, a comprehensive understanding of the role of the cytoskeleton in erythrocyte enucleation is of paramount importance for elucidating the cellular mechanisms underlying erythrocyte production, understanding the pathogenesis of erythrocyte-related hematological disorders, and developing novel therapeutic strategies [46]. Future research may reveal novel therapeutic targets to enhance erythrocyte production in conditions characterized by ineffective erythropoiesis.

3.4 | Non-coding RNA regulation of the enucleation process

During erythrocyte enucleation, various non-coding RNAs play critical regulatory roles. miR-191 was the first microRNA reported to regulate the enucleation of erythroblasts in the fetal liver of mice [49]. Its downregulation during enucleation leads to the upregulation of target genes Riok3 and Mxi1, which are associated with the antagonistic action of Gcn5, ultimately promoting erythrocyte enucleation [50]. Additionally, several microRNAs, such as miR-30a, miR-34a, and miR-9, have been found to inhibit enucleation during the differentiation of erythrocytes derived from human embryonic stem cells (hESCs), while miR-181a suppresses normal erythrocyte enucleation by inhibiting Xpo7 [51, 52]. Moreover, the significance of long non-coding RNAs (lncRNAs) in erythropoiesis is increasingly recognized. For instance, Bloodlinc is a lncRNA transcribed from the super-enhancer of the erythrocyte membrane transporter gene SLC4A1/BAND3 and is located at a chromosomal locus that encodes key regulatory factors and effectors of terminal erythropoiesis [53]. Bloodlinc plays a pivotal role in erythrocyte enucleation by physically interacting with the chromatin-binding protein HNRNPU, stabilizing RNA-chromatin interactions. Inhibition of either Bloodlinc or HNRNPU significantly impairs the enucleation process of fetal liver erythrocytes [53].

Non-coding RNAs exhibit complex and essential regulatory functions in erythropoiesis, not only participating in the fine-tuning of cell differentiation and proliferation but also being involved in the physical enucleation of the nucleus. These findings provide new insights into the molecular mechanisms underlying erythropoiesis and offer potential therapeutic targets for related diseases.

3.5 | Autophagy and ubiquitin-proteasome degradation in the process of erythrocyte enucleation

Autophagy and protein degradation effectively regulate the process of erythrocyte enucleation. As an internal cellular housekeeping mechanism, autophagy not only removes oxidized or damaged proteins but also eliminates structures such as mitochondria that produce large amounts of reactive oxygen species (ROS), thereby reducing further ROS generation and playing a regulatory role in erythrocyte enucleation [54]. Meanwhile, ROS and the modifications they induce on signaling proteins play key roles during erythrocyte enucleation, and the use of antioxidants to protect erythrocytes from oxidative stress is one of the key factors for promoting enucleation in vitro [54]. Autophagy is particularly crucial in erythrocyte enucleation, with mitophagy playing a key role in the removal of mitochondria during the enucleation of reticulocytes, mediated by mitophagy receptors such as BCL2-interacting protein 3-like (BNIP3L/Nix) [55, 56]. These receptors tether autophagosomes to the mitochondrial outer membrane to mediate mitochondrial clearance, while factors like FoxO3 indirectly regulate mitophagy by controlling the expression of autophagy-related genes such as Nix [57]. Additionally, FoxO3 regulates mitochondrial depolarization and mitophagy by modulating PTEN-induced kinase 1 (PINK1). Thus, as a critical regulator of autophagy, FoxO3 greatly facilitates the erythrocyte enucleation process [58]. Betin et al.'s research specifically revealed the regulatory mechanisms of autophagy and its influence on erythroid differentiation during maturation. By overexpressing specific cysteine mutants (C74A and C144A) of ATG4B and ATG4D, these mutants formed stable complexes with members of the ATG8 family, thereby blocking the progress of autophagy. Despite limiting autophagosome formation, erythrocytes were able to compensate for the impaired autophagic process through alternative pathways, such as non-ATG8-dependent autophagosome assembly (e.g., ATG5/ATG7-independent and RAB9-dependent pathways). Furthermore, these ATG4 cysteine mutants also affected autophagosome maturation, particularly by preventing the normal fusion and degradation processes of specific proteins such as LAMP1, leading to the accumulation of undegraded cytoplasmic material. Despite these disruptions to the autophagic process, the study demonstrated that erythroid differentiation dynamics remained normal. These findings highlight the importance of autophagy in erythroid maturation and reveal how erythrocytes maintain their normal differentiation capacity even when the autophagic process is impaired [59]. By selectively degrading organelles, autophagy preserves cellular integrity, making it a crucial step in the transition from reticulocytes to mature erythrocytes. Moreover, protein degradation pathways such as the ubiquitin-proteasome system (UPS) play indispensable roles in regulating cell cycle progression and cytoskeleton reorganization, both of which are essential for the successful expulsion of the erythrocyte nucleus. The UPS regulates protein degradation via polyubiquitination. Ubiquitination and its associated ubiquitin-conjugating enzymes function at various stages of erythropoiesis, with specific upregulation of E2 ubiquitin-conjugating enzymes such as E2-20K and E2-230K in reticulocytes [60]. Ubiquitin ligases Mdm2 and Mdm4 regulate erythrocyte enucleation by antagonizing p53, while the E3 ubiquitin ligase Cul4A promotes erythroid progenitor proliferation by targeting p27 for degradation [61]. Research on the ubiquitin-conjugating enzyme UBE2O has shown that ubiquitin-mediated degradation of ribosomal proteins and other non-ribosomal targets such as histone H2B can reshape the proteome in reticulocytes [62].

The synergistic action of autophagy and the UPS in the process of erythrocyte enucleation is not only crucial for maintaining erythrocyte health but also provides valuable insights and potential therapeutic strategies for understanding and treating erythropoiesis-related diseases. Future research in this area is expected to offer new perspectives on the molecular mechanisms governing erythrocyte enucleation and provide novel approaches and strategies for treating related diseases.

4 | IN VITRO ERYTHROPOIESIS

4.1 | Induced enucleation for erythrocyte production

Although in vitro simulation of enucleation remains a challenge, numerous studies have revealed various genes involved in the enucleation process, providing a strong theoretical basis for laboratory-cultured mature erythrocytes. Distinguishing direct or indirect factors affecting enucleation is complex, as decreased enucleation efficiency under conditions of functional impairment may be due to upstream stages of erythrocyte production being compromised [63, 64]. Consequently, many researchers have attempted to identify exogenous factors that can induce enucleation or effectively improve conditions for in vitro erythrocyte differentiation. EBI represents a multicellular structure within the erythroid microenvironment of the bone marrow, consisting of a central macrophage and surrounding erythrocytes at different maturation stages. Despite the recognized importance of macrophages and key proteins like macrophage-erythroid attacher, their specific roles in triggering the enucleation process remain unclear [65]. EPO is the primary factor for erythrocyte production, but its role in enucleation is yet to be elucidated, while high levels of EPO are known to reduce enucleation efficiency by inducing oxidative stress [66]. To identify key factors triggering enucleation, a series of gene expression and proteomic analyses have been conducted. These studies are particularly crucial for in vitro erythrocyte generation as laboratory-cultured erythrocytes are considered a promising alternative to the existing donor-dependent transfusion system [67]. Hematopoietic stem and progenitor cells (HSPCs) serve as the source for all blood lineage cells, and thus, early cells isolated from umbilical cord blood (UCB) or peripheral blood have been extensively studied as sources for in vitro erythrocyte production [68]. These approaches have demonstrated efficacy in enucleation rates, with final-stage efficiencies reaching approximately 70%–80%. Nevertheless, more sustainable and stable sources are still required, considering that a healthy adult produces approximately 2.5 million erythrocytes per second, demanding substantial resources and technology [68, 69]. To address the issue of low enucleation rates, scientists have conducted a series of studies. Lapillonne and colleagues first used a suspension culture of hEBs to successfully differentiate human induced pluripotent stem cells (hiPSCs) into RBCs. This pioneering work, while groundbreaking, resulted in RBCs with an enucleation rate of only 4%–10%, significantly lower than the 30–65% rate achieved with hESCs [70]. By inhibiting miR30A, they were able to increase the enucleation rate to 50%, while ensuring that the functional RBCs could express both fetal and adult hemoglobins [50]. Additionally, research by Kobari and others further demonstrated that their hiPSCs could achieve an enucleation rate of 20%–26% in NOD/SCID mice and successfully synthesize adult hemoglobin. Subsequent studies focused on increasing the proliferation and enucleation rates of RBCs, and on transitioning this process from small-scale suspension cultures to larger-scale production [71]. Olivier and colleagues found that by introducing various novel small molecules (such as FGF, HPO, TPO, insulin, etc.) to promote the proliferation of erythroid-like cells, the EB differentiation process could be optimized to some extent, improving the enucleation efficiency to 10% [72]. In the same year, Sivalingam and his team established a comprehensive amplification process for multiple hiPSC lines in 1L bioreactors, increasing the enucleation rate to 15%. By modulating the Wnt/β-Catenin signaling pathway and co-culturing with human mesenchymal stem cells (hMSCs), successfully amplified erythroid cells by 10,000 times, achieving enucleation rates of 28%–40.6% [73, 74]. They further developed a method for scalable differentiation of hiPSC microcarrier aggregates in 500 mL bioreactors, significantly increasing the proliferation rate of functional RBCs. Although the direct enucleation rate was only 6%, co-culturing with OP9 cells increased the in vitro enucleation rate to 59.3%. Recent research efforts to further increase enucleation rates have included the addition of new components and small molecules [75]. Bernecker and others successfully formed hematopoietic cell-forming complexes (HCFCs) by mimicking the physiological niche of erythroid cells, achieving enucleation rates of 40% to 60% [74]. Yrigoyen and colleagues found that KLF1-activated hiPSC-derived macrophages (iPSC-DMs) could enhance the enucleation of RBC populations. They identified three secretory factors (ANGPTL7, interleukin [IL]-33, and SERPINB2) sufficient to replace the feeder layer, leading to an in vitro enucleation rate of up to 73% [76]. These studies reveal that promoting enucleation is a key method for future in vitro RBC generation. Here, we summarize different strategies to promote erythrocyte enucleation (Table 1).

4.2 | Regulatory strategies for HSPC-driven erythropoiesis

Ex vivo maintenance and expansion of human HSPCs are critical areas in hematology and regenerative medicine research, especially for improving hematopoietic stem cell transplantation (HSCT) outcomes and developing gene therapy strategies [77]. However, stable expansion of hematopoietic stem cells (HSCs) ex vivo remains a significant challenge. The ex vivo maintenance and expansion of human HSPCs are primarily influenced by their signaling pathways, cytokine effects, co-culture system development, and the use of small molecules [78]. Various cytokines have been demonstrated to support the ex vivo maintenance and expansion of HSPCs. The most widely used combination includes stem cell factor (SCF), thrombopoietin (THPO), Fms-like tyrosine kinase 3 ligand (FLT3L), and IL-6 [79–81]. SCF has been found to promote HSC survival and proliferation by activating the PI3K/AKT/FOXO signaling pathway [78]. THPO, through

TABLE 1 Strategies and efficiencies in enhancing erythrocyte enucleation.

Researcher name	Study project	Efficiency	Strategy or challenge	Ref
Lapillonne et al.	Differentiation of erythrocytes from hEB suspension culture	4%–10%	Using hEB suspension culture for differentiation	[70]
Rouzbeh et al.	Enhancing the enucleation rate	~50%	Enhancing the enucleation rate	[50]
Kobari et al.	Achieving enucleation and adult hemoglobin synthesis in NOD/SCID mice	20%–26%	Enucleation in NOD/SCID mice	[71]
Olivier et al.	Erythroid-like cell expansion	10%	Introducing various novel small molecules (FGF, HPO, TPO, insulin, etc.)	[72]
Sivalingam et al.	Improving in vitro enucleation rate	Up to 59.3%	Improving in vitro enucleation rate	[73]
Bernecker et al.	Forming HCFCs to mimic the erythroid niche	40%–60%	Mimicking the erythroid physiological niche	[74]
Yrigoyen et al.	IPSC-DMs	73%	KLF1-activated hiPSC-DMs	[76]

Note: Table 1 summarizes strategies to enhance erythrocyte enucleation rates in vitro, their efficiencies, and challenges, based on published studies. Reference numbers are provided for source verification.

its binding to the MPL receptor, can activate the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) and PI3K/protein kinase B (PI3K/AKT) signaling pathways, maintaining HSC survival. FLT3L stimulates HSPC growth by activating the MAPK/ERK and JAK/STAT3/5 pathways [82]. IL-6, in conjunction with its receptor IL-6R, activates the JAK1/JAK2/TYK2 signaling pathway and promotes SRC kinase expression, thereby facilitating HSPC expansion [83]. Promoting ex vivo HSC survival and expansion through targeting key molecules or delivering their inhibitors has been the focus of many studies, some of which have entered clinical trials with promising results. Tetraethylenepentamine (TEPA): TEPA promotes the differentiation and proliferation of UCB-derived HSPCs by chelating copper ions, leading to expanded HSPC proliferation and enhanced repopulation abilities in immunodeficient NSG mice. Clinical trials have shown that TEPA can increase cell proliferation rates and accelerate hematopoietic recovery [84]. StemRegenin-1 (SR1): SR1, a purine derivative that inhibits the aryl hydrocarbon receptor, significantly increases the expansion of UCB-derived HSPCs and the number of SRCs, with clinical trials observing rapid hematopoietic recovery and enhanced engraftment capabilities [85]. However, issues related to high transplant-related mortality and low overall survival rates need further resolution. Nicotinamide (NAM): NAM enhances the homing of CXCR4-CXCL12-based UCB-derived CD34+ HSPCs, significantly improving their expansion and implantation potential. Clinical trials have shown that the use of NAM-expanded UCB units results in a lower rate of long-term engraftment failure and higher survival rates [86]. Prostaglandin E2 (PGE2): PGE2 and its stable derivative dmPGE2 improve HSC self-renewal and engraftment capabilities in NSG mice by activating the Wnt pathway. Endogenous regulation of PGE2 levels is considered an attractive method for improving HSCT efficacy, with clinical trials using dmPGE2 showing rapid hematopoietic recovery and higher long-term engraftment rates [87]. UM729 and UM171, two pyrimidoindole derivatives, inhibit genes associated with erythrocyte and megakaryocyte differentiation by affecting LSD1, allowing for efficient expansion and maintenance of long-term repopulating abilities of HSPCs [88, 89]. Clinical trials of UM171 have shown high survival rates without transplantation failure, and ongoing trials aim to further assess its safety and effectiveness. MAPK inhibitors: MAPK inhibitors represent a promising strategy for promoting HSPC expansion. Using SB203580 to inhibit p38 MAPK has been shown to enhance the expansion, self-renewal, and re-engraftment capabilities of UCB-derived HSPCs. Analogs of SB203580, such as C7, have shown enhanced HSC expansion activity and promoted long-term reconstruction capabilities in primary and secondary NSG mouse recipients [90]. Additionally, the use of JNK-IN-8 has been able to expand human UCB HSPCs, significantly increasing SRC frequency in primitive recipients and achieving engraftment in secondary recipients [91]. The significance of HSPCs' erythropoiesis method lies in providing a novel approach to promote RBC production by regulating stem cell self-renewal and proliferation capabilities, with the potential to improve treatment outcomes for conditions such as anemia. This method holds promising prospects for offering more effective therapeutic interventions for patients and advancing research and clinical applications in the field (Table 2).

5 | CONCLUSION

In this review, we delve into various aspects of the regulatory mechanisms underlying chromatin condensation and the processes associated with erythrocyte enucleation. Additionally, we provide a comprehensive summary of studies on UPS, autophagy, and other related factors, enhancing our understanding of the molecular mechanisms involved in erythrocyte enucleation. ESCs and iPS cells have been widely used as starting materials in numerous fields of erythrocyte enucleation research and application. Their unlimited proliferative potential and capacity to differentiate into any cell type make them ideal candidates for in vitro erythrocyte production. iPS cells, in particular, derived from specific patients and reprogrammed, offer the

TABLE 2 Therapeutic compounds for hematopoietic stem cell (HSC) expansion and enhancement.

Drug/Compound	Mechanism of Action	Effect/Application	Features	Ref.
Tetraethylenepentamine (TEPA)	Chelates copper ions	Enhanced proliferation and reconstitution abilities in HSPCs	Used for UCB-derived HSPCs	[84]
StemRegenin-1 (SR1)	Inhibits the aryl hydrocarbon receptor (AHR)	Rapid hematopoietic recovery and enhanced engraftment capabilities	UCB-derived HSPCs expansion	[85]
Nicotinamide (NAM)	Enhances homing of CXCR4-CXCL12-based HSPCs	Significant improvement in expansion and engraftment potential	UCB CD34+ HSPCs expansion	[86]
Prostaglandin E2 (PGE2)	Activates the Wnt pathway	Improves HSC self-renewal and engraftment capabilities	Endogenous regulation of PGE2 levels	[87]
UM729 and UM171	Affects LSD1-inhibiting genes related to erythrocyte and megakaryocyte differentiation	Efficient expansion and maintenance of long-term repopulating abilities	High survival rates without transplantation failure	[88, 89]

Note: Table 2 summarizes key therapeutic compounds and their respective mechanisms of action that have been studied for enhancing the expansion and functional capabilities of hematopoietic stem cells (HSCs).

significant advantage of avoiding immune rejection, making them especially suitable for autologous transfusions. Furthermore, the development of immortalized erythroid cell lines provides an intriguing alternative. Immortalized erythroid cell lines driven by human oncogenic viral genes E6/E7 have demonstrated the potential for sustained erythrocyte proliferation in vitro. Although these immortalized cell lines exhibit lower enucleation efficiency, they offer an effective platform for studying erythroid differentiation and maturation mechanisms. Several studies are currently exploring ways to improve the enucleation efficiency and functionality of erythrocytes derived from these sources. For instance, improvements in culture conditions and modifications to differentiation protocols have successfully enhanced the enucleation rates of iPS cell-derived erythrocytes. These studies often utilize feeder-free systems or co-cultures with specific cell lines, such as MS5 or MSC. In terms of clinical applications and commercialization, key challenges include scaling up the production of high-quality enucleated erythrocytes, ensuring their functionality resembles that of natural erythrocytes, and controlling production costs. It is also essential to ensure that these in vitro-cultured erythrocytes can function safely and effectively within the human body without eliciting immune responses or other adverse effects. Future research directions may include the development of novel cell engineering technologies to optimize the processes of erythrocyte production and maturation, as well as exploring new cell sources and bioreactor technologies to achieve the industrial-scale production of erythrocytes. As our understanding of erythrocyte biology deepens, new therapeutic targets may be identified, further enhancing the efficacy and safety of treatments.

ACKNOWLEDGMENTS

This research was supported by the National Key Research and Development Program of China (grant number 2022YFC2406803) and the National Natural Science Foundation of China (Grant nos. 82070114, and 82270126).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

This review article does not contain any new data. It is a synthesis and analysis of previously published literature and existing research. Therefore, there are no new datasets generated in this study that require sharing or availability.

ETHICS STATEMENT

The authors have confirmed ethical approval statement is not needed for this submission.

PATIENT CONSENT STATEMENT

The authors have confirmed patient consent statement is not needed for this submission.

CLINICAL TRIAL REGISTRATION

The authors have confirmed clinical trial registration is not needed for this submission.

ORCID

Qianli Zhuo https://orcid.org/0009-0006-2040-7133
Zhaojun Zhang https://orcid.org/0000-0003-0490-6507
Xiangdong Fang https://orcid.org/0000-0002-6628-8620

REFERENCES

1. Tormey CA, Hendrickson JE. Transfusion-related red blood cell alloantibodies: induction and consequences. Blood. 2019;133:1821–30. DOI: 10.1182/blood-2018-08-833962
2. Jin X, Zhang Y, Wang D, Zhang X, Li Y, Wang D, et al. Metabolite and protein shifts in mature erythrocyte under hypoxia. iScience. 2024;27:109315. DOI: 10.1016/j.isci.2024.109315
3. Zivot A, Lipton JM, Narla A, Blanc L. Erythropoiesis: insights into pathophysiology and treatments in 2017. Mol Med. 2018;24:11. DOI: 10.1186/s10020-018-0011-z
4. Caulier A, Guyonneau Harmand L, Garçon L. Immortalisation érythrocytaire pour production de globules rouges in vitro. Transfus Clin Biol. 2017;24:263–67. DOI: 10.1016/j.tracli.2017.06.030
5. Fujii J, Homma T, Kobayashi S, Warang P, Madkaikar M, Mukherjee MB. Erythrocytes as a preferential target of oxidative stress in blood.

Free Radic Res. 2021;55:781–99. DOI: 10.1080/10715762.2021.1873318
6. Soboleva S, Miharada K. Induction of enucleation in primary and immortalized erythroid cells. Int J Hematol. 2022;116:192–98. DOI: 10.1007/s12185-022-03386-w
7. Weidemann A, Johnson RS. Nonrenal regulation of EPO synthesis. Kidney Int. 2009;75:682–88. DOI: 10.1038/ki.2008.687
8. Zhao B, Yang J, Ji P. Chromatin condensation during terminal erythropoiesis. Nucleus. 2016;7:425–29. DOI: 10.1080/19491034.2016.1226717
9. Menon V, Ghaffari S. Erythroid enucleation: a gateway into a "bloody" world. Exp Hematol. 2021;95:13–22. DOI: 10.1016/j.exphem.2021.01.001
10. Ren K, Li E, Ji P. Proteome remodeling and organelle clearance in mammalian terminal erythropoiesis. Curr Opin Hematol. 2022;29:137–43. DOI: 10.1097/MOH.0000000000000707
11. Ovchynnikova E, Aglialoro F, Von Lindern M, Van Den Akker E. The shape shifting story of reticulocyte maturation. Front Physiol. 2018;9:829. DOI: 10.3389/fphys.2018.00829
12. Zweig SE, Tokuyasu KT, Singer SJ. Member-associated changes during erythropoiesis. On the mechanism of maturation of reticulocytes to erythrocytes. J Supramol Struct Cell Biochem. 1981;17:163–81. DOI: 10.1002/jsscb.380170207
13. Mei Y, Liu Y, Ji P. Understanding terminal erythropoiesis: an update on chromatin condensation, enucleation, and reticulocyte maturation. Blood Rev. 2021;46:100740. DOI: 10.1016/j.blre.2020.100740
14. Wang S, Zhao H, Zhang H, Gao C, Guo X, Chen L, et al. Analyses of erythropoiesis from embryonic stem cell-CD34+ and cord blood-CD34+ cells reveal mechanisms for defective expansion and enucleation of embryonic stem cell-erythroid cells. J Cell Mol Med. 2022;26:2404–16. DOI: 10.1111/jcmm.17263
15. Jayapal SR, Lee KL, Ji P, Kaldis P, Lim B, Lodish HF. Down-regulation of Myc is essential for terminal erythroid maturation. J Biol Chem. 2010;285:40252–65. DOI: 10.1074/jbc.M110.181073
16. Rossmann MP, Hoi K, Chan V, Abraham BJ, Yang S, Mullahoo J, et al. Cell-specific transcriptional control of mitochondrial metabolism by TIF1γ drives erythropoiesis. Science. 2021;372:716–21. DOI: 10.1126/science.aaz2740
17. Bhoopalan SV, Huang LJ-S, Weiss MJ. Erythropoietin regulation of red blood cell production: from bench to bedside and back. F1000Res. 2020;9:1153. DOI: 10.12688/f1000research.26648.1
18. Semenza GL. Regulation of erythropoiesis by the hypoxia-inducible factor pathway: effects of genetic and pharmacological perturbations. Annu Rev Med. 2023;74:307–19. DOI: 10.1146/annurev-med-042921-102602
19. Tian Z, Yao Lv, Shen Y, Guo X, Duan X. Histone H3K9 demethylase JMJD1A is a co-activator of erythropoietin expression under hypoxia. Int J Biochem Cell Biol. 2019;109:33–39. DOI: 10.1016/j.biocel.2019.01.022
20. Carlile GW, Smith DH, Wiedmann M. Caspase-3 has a nonapoptotic function in erythroid maturation. Blood. 2004;103:4310–16. DOI: 10.1182/blood-2003-09-3362
21. Zhao B, Mei Y, Schipma MJ, Roth EW, Bleher R, Rappoport JZ, et al. Nuclear condensation during mouse erythropoiesis requires caspase-3-mediated nuclear opening. Dev Cell. 2016;36:498–510. DOI: 10.1016/j.devcel.2016.02.001
22. Zhen Ru, Moo C, Zhao Z, Chen M, Feng He, Zheng X, et al. Wdr26 regulates nuclear condensation in developing erythroblasts. Blood. 2020;135:208–19. DOI: 10.1182/blood.2019002165
23. Delehanty LL, Bullock GC, Goldfarb AN. Protein kinase D-HDAC5 signaling regulates erythropoiesis and contributes to erythropoietin cross-talk with GATA1. Blood. 2012;120:4219–28. DOI: 10.1182/blood-2011-10-387050
24. Varricchio L, Dell'aversana C, Nebbioso A, Migliaccio G, Altucci L, Mai A, et al. Identification of NuRSERY, a new functional HDAC complex composed by HDAC5, GATA1, EKLF and pERK present in human erythroid cells. Int J Biochem Cell Biol. 2014;50:112–22. DOI: 10.1016/j.biocel.2014.02.019
25. Kapoor-Vazirani P, Kagey J, Vertino P. SUV420H2-mediated H4K20 trimethylation enforces RNA polymerase II promoter-proximal pausing by blocking hMOF-dependent H4K16 acetylation. Mol Cell Biol. 2011;31:1594–609. DOI: 10.1128/MCB.00524-10
26. Romano L, Seu KG, Papoin J, Muench DE, Konstantinidis D, Olsson A, et al. Erythroblastic islands foster granulopoiesis in parallel to terminal erythropoiesis. Blood. 2022;140:1621–34. DOI: 10.1182/blood.2022015724
27. Salomao M, Chen Ke, Villalobos J, Mohandas N, An X, Chasis JA. Hereditary spherocytosis and hereditary elliptocytosis: aberrant protein sorting during erythroblast enucleation. Blood. 2010;116:267–69. DOI: 10.1182/blood-2010-02-264127
28. Ji P, Lodish HF. Ankyrin and band 3 differentially affect expression of membrane glycoproteins but are not required for erythroblast enucleation. Biochem Biophys Res Commun. 2012;417:1188–92. DOI: 10.1016/j.bbrc.2011.12.105
29. Stevens-Hernandez CJ, Bruce LJ. Reticulocyte Maturation. Membranes. 2022;12:311. DOI: 10.3390/membranes12030311
30. Lessard S, Beaudoin M, Benkirane K, Lettre G. Comparison of DNA methylation profiles in human fetal and adult red blood cell progenitors. Genome Med. 2015;7:1. DOI: 10.1186/s13073-014-0122-2
31. Hattangadi SM, Wong P, Zhang L, Flygare J, Lodish HF. From stem cell to red cell: regulation of erythropoiesis at multiple levels by multiple proteins, RNAs, and chromatin modifications. Blood. 2011;118:6258–68. DOI: 10.1182/blood-2011-07-356006
32. Lv X, Murphy K, Murphy Z, Getman M, Rahman N, Nakamura Y, et al. HEXIM1 is an essential transcription regulator during human erythropoiesis. Blood. 2023;142:2198–215. DOI: 10.1182/blood.2022019495
33. Lu Z, Huang L, Li Y, Xu Y, Zhang R, Zhou Q, et al. Fine-tuning of cholesterol homeostasis controls erythroid differentiation. Adv Sci. 2021;9:e2102669. DOI: 10.1002/advs.202102669
34. Condorelli G, Vitelli L, Valtieri M, Marta I, Montesoro E, Lulli V, et al. Coordinate expression and developmental role of Id2 protein and TAL1/E2A heterodimer in erythroid progenitor differentiation. Blood. 1995;86:164–75. DOI: 10.1182/blood.V86.1.164.bloodjournal861164
35. Liu D, Duan L, Rodda LB, Lu E, Xu Y, An J, et al. CD97 promotes spleen dendritic cell homeostasis through the mechanosensing of red blood cells. Science. 2022;375:eabi5965. DOI: 10.1126/science.abi5965
36. Li D, Wu F, Zhou S, Huang X-J, Lee H-Y. Heterochromatin rewiring and domain disruption-mediated chromatin compaction during erythropoiesis. Nat Struct Mol Biol. 2023;30:463–74. DOI: 10.1038/s41594-023-00939-3
37. Bakker WJ, Van Dijk TB, Parren-Van Amelsvoort M, Kolbus A, Yamamoto K, Steinlein P, et al. Differential regulation of Foxo3a target genes in erythropoiesis. Mol Cell Biol. 2007;27:3839–54. DOI: 10.1128/MCB.01662-06
38. Bakker WJ, Blázquez-Domingo M, Kolbus A, Besooyen J, Steinlein P, Beug H, et al. FoxO3a regulates erythroid differentiation and induces BTG1, an activator of protein arginine methyl transferase 1. J Cell Biol. 2004;164:175–84. DOI: 10.1083/jcb.200307056
39. Liang R, Campreciós G, Kou Y, Mcgrath K, Nowak R, Catherman S, et al. A systems approach identifies essential FOXO3 functions at key steps of terminal erythropoiesis. PLoS Genet. 2015;11:e1005526. DOI: 10.1371/journal.pgen.1005526
40. Koury ST, Koury MJ, Bondurant MC. Cytoskeletal distribution and function during the maturation and enucleation of mammalian erythroblasts. J Cell Biol. 1989;109:3005–13. DOI: 10.1083/jcb.109.6.3005

41. Pollard TD, Wu J-Q. Understanding cytokinesis: lessons from fission yeast. Nat Rev Mol Cell Biol. 2010;11:149–55. DOI: 10.1038/nrm2834
42. Wang J, Ramirez T, Ji P, Jayapal SR, Lodish HF, Murata-Hori M. Mammalian erythroblast enucleation requires PI3K-dependent cell polarization. J Cell Sci. 2012;125:340–49. DOI: 10.1242/jcs.088286
43. Ji P. New insights into the mechanisms of mammalian erythroid chromatin condensation and enucleation. Int Rev Cell Mol Biol. 2015;316:159–82. DOI: 10.1016/bs.ircmb.2015.01.006
44. Ji P, Jayapal SR, Lodish HF. Enucleation of cultured mouse fetal erythroblasts requires Rac GTPases and mDia2. Nat Cell Biol. 2008;10:314–21. DOI: 10.1038/ncb1693
45. Mei Y, Zhao B, Yang J, Gao J, Wickrema A, Wang D, et al. Ineffective erythropoiesis caused by binucleated late-stage erythroblasts in mDia2 hematopoietic specific knockout mice. Haematologica. 2015;101:e1–e5. DOI: 10.3324/haematol.2015.134221
46. Fraser ST, Midwinter RG, Coupland LA, Kong S, Berger BS, Yeo JH, et al. Heme oxygenase-1 deficiency alters erythroblastic island formation, steady-state erythropoiesis and red blood cell lifespan in mice. Haematologica. 2015;100:601–10. DOI: 10.3324/haematol.2014.116368
47. Perkins A, Xu X, Higgs DR, Patrinos GP, Arnaud L, Bieker JJ, et al. Krüppeling erythropoiesis: an unexpected broad spectrum of human red blood cell disorders due to KLF1 variants. Blood. 2016;127:1856–62. DOI: 10.1182/blood-2016-01-694331
48. Zhao B, Keerthivasan G, Mei Y, Yang J, Mcelherne J, Wong P, et al. Targeted shRNA screening identified critical roles of pleckstrin-2 in erythropoiesis. Haematologica. 2014;99:1157–67. DOI: 10.3324/haematol.2014.105809
49. Zhang L, Flygare J, Wong P, Lim B, Lodish HF. miR-191 regulates mouse erythroblast enucleation by down-regulating Riok3 and Mxi1. Genes Dev. 2010;25:119–24. DOI: 10.1101/gad.1998711
50. Rouzbeh S, Kobari L, Cambot M, Mazurier C, Hebert N, Faussat A-M, et al. Molecular signature of erythroblast enucleation in human embryonic stem cells. Stem Cells. 2015;33:2431–41. DOI: 10.1002/stem.2027
51. Figueroa AA, Fasano JD, Martinez-Morilla S, Venkatesan S, Kupfer G, Hattangadi SM. miR-181a regulates erythroid enucleation via the regulation of Xpo7 expression. Haematologica. 2018;103:e341–44. DOI: 10.3324/haematol.2017.171785
52. Alvarez-Dominguez JR, Lodish HF. Emerging mechanisms of long noncoding RNA function during normal and malignant hematopoiesis. Blood. 2017;130:1965–75. DOI: 10.1182/blood-2017-06-788695
53. Alvarez-Dominguez JR, Knoll M, Gromatzky AA, Lodish HF. The super-enhancer-derived alncRNA-EC7/Bloodlinc potentiates red blood cell development in trans. Cell Rep. 2017;19:2503–14. DOI: 10.1016/j.celrep.2017.05.082
54. Zhou J, Li X-Yu, Liu Yu-J, Feng Ji, Wu Y, Shen H-M, et al. Full-coverage regulations of autophagy by ROS: from induction to maturation. Autophagy. 2021;18:1240–55. DOI: 10.1080/15548627.2021.1984656
55. Sandoval H, Thiagarajan P, Dasgupta SK, Schumacher A, Prchal JT, Chen M, et al. Essential role for Nix in autophagic maturation of erythroid cells. Nature. 2008;454:232–35. DOI: 10.1038/nature07006
56. Schweers RL, Zhang Ji, Randall MS, Loyd MR, Li W, Dorsey FC, et al. NIX is required for programmed mitochondrial clearance during reticulocyte maturation. Proc Natl Acad Sci. 2007;104:19500–19505. DOI: 10.1073/pnas.0708818104
57. Zhu M, Du L, Zhao R, Wang HY, Zhao Y, Nie G, et al. Cell-penetrating nanoparticles activate the inflammasome to enhance antibody production by targeting microtubule-associated protein 1-light chain 3 for degradation. ACS Nano. 2020;14:3703–17. DOI: 10.1021/acsnano.0c00962
58. Jin SM, Youle RJ. PINK1- and Parkin-mediated mitophagy at a glance. J Cell Sci. 2012;125:795–99. DOI: 10.1242/jcs.093849
59. Betin VMS, Singleton BK, Parsons SF, Anstee DJ, Lane JD. Autophagy facilitates organelle clearance during differentiation of human erythroblasts. Autophagy. 2013;9:881–93. DOI: 10.4161/auto.24172
60. Wefes I, Mastrandrea LD, Haldeman M, Koury ST, Tamburlin J, Pickart CM, et al. Induction of ubiquitin-conjugating enzymes during terminal erythroid differentiation. Proc Natl Acad Sci. 1995;92:4982–86. DOI: 10.1073/pnas.92.11.4982
61. Maetens M, Doumont G, Clercq SD, Francoz S, Froment P, Bellefroid E, et al. Distinct roles of Mdm2 and Mdm4 in red cell production. Blood. 2007;109:2630–33. DOI: 10.1182/blood-2006-03-013656
62. Nguyen AT, Prado MA, Schmidt PJ, Sendamarai AK , Wilson-Grady JT, Min M, et al. UBE2O remodels the proteome during terminal erythroid differentiation. Science. 2017;357. DOI: 10.1126/science.aan0218
63. Chasis JA. Erythroblastic islands: specialized microenvironmental niches for erythropoiesis. Curr Opin Hematol. 2006;13:137–41. DOI: 10.1097/01.moh.0000219657.57915.30
64. Giger KM, Kalfa TA. Phylogenetic and ontogenetic view of erythroblastic islands. Biomed Res Int. 2015;2015:873628. DOI: 10.1155/2015/873628
65. Zhao B, Mei Y, Yang J, Ji P. Erythropoietin-regulated oxidative stress negatively affects enucleation during terminal erythropoiesis. Exp Hematol. 2016;44:975–81. DOI: 10.1016/j.exphem.2016.06.249
66. Bell AJ, Satchwell TJ, Heesom KJ, Hawley BR, Kupzig S, Hazell M, et al. Protein distribution during human erythroblast enucleation in vitro. PLoS One. 2013;8:e60300. DOI: 10.1371/journal.pone.0060300
67. Peng Y, Tang Li, Li Y, Song J, Liu H, Wang P, et al. Comprehensive proteomic analysis reveals dynamic phospho-profiling in human early erythropoiesis. Br J Haematol. 2022;199:427–42. DOI: 10.1111/bjh.18407
68. Giarratana M-C, Kobari L, Lapillonne H, Chalmers D, Kiger L, Cynober T, et al. Ex vivo generation of fully mature human red blood cells from hematopoietic stem cells. Nat Biotechnol. 2005;23:69–74. DOI: 10.1038/nbt1047
69. Neildez-Nguyen TMyA, Wajcman H, Marden MC, Bensidhoum M, Moncollin V, Giarratana M-C, et al. Human erythroid cells produced ex vivo at large scale differentiate into red blood cells in vivo. Nat Biotechnol. 2002;20:467–72. DOI: 10.1038/nbt0502-467
70. Lapillonne H, Kobari L, Mazurier C, Tropel P, Giarratana M-C, Zanella-Cleon I, et al. Red blood cell generation from human induced pluripotent stem cells: perspectives for transfusion medicine. Haematologica. 2010;95:1651–59. DOI: 10.3324/haematol.2010.023556
71. Kobari L, Yates F, Oudrhiri N, Francina A, Kiger L, Mazurier C, et al. Human induced pluripotent stem cells can reach complete terminal maturation: in vivo and in vitro evidence in the erythropoietic differentiation model. Haematologica. 2012;97:1795–803. DOI: 10.3324/haematol.2011.055566
72. Olivier EN, Marenah L, Mccahill A, Condie A, Cowan S, Mountford JC. High-efficiency serum-free feeder-free erythroid differentiation of human pluripotent stem cells using small molecules. Stem Cells Transl Med. 2016;5:1394–405. DOI: 10.5966/sctm.2015-0371
73. Sivalingam J, Chen HYu, Yang B-X, Lim ZRi, Lam ATL, Woo TL, et al. Improved erythroid differentiation of multiple human pluripotent stem cell lines in microcarrier culture by modulation of Wnt/β-Catenin signaling. Haematologica. 2018;103:e279–83. DOI: 10.3324/haematol.2017.180919
74. Bernecker C, Ackermann M, Lachmann N, Rohrhofer L, Zaehres H, Araúzo-Bravo MJ, et al. Enhanced ex vivo generation of erythroid cells from human induced pluripotent stem cells in a simplified cell culture system with low cytokine support. Stem Cells Dev. 2019;28: 1540–51. DOI: 10.1089/scd.2019.0132
75. Sivalingam J, Sue Yu, Lim ZRi, Lam ATL, Lee AP, Lim HL, et al. A scalable suspension platform for generating high-density cultures of universal red blood cells from human induced pluripotent stem cells. Stem Cell Rep. 2021;16:182–97. DOI: 10.1016/j.stemcr.2020.11.008

76. Lopez-Yrigoyen M, Yang C-T, Fidanza A, Cassetta L, Taylor AH, Mccahill A, et al. Genetic programming of macrophages generates an in vitro model for the human erythroid island niche. Nat Commun. 2019;10:881. DOI: 10.1038/s41467-019-08705-0
77. Tang P, Wang H. Regulation of erythropoiesis: emerging concepts and therapeutic implications. Hematology. 2023;28:2250645. DOI: 10.1080/16078454.2023.2250645
78. Lim ZRi, Vassilev S, Leong YW, Hang JW, Rénia L, Malleret B, et al. Industrially compatible transfusable iPSC-derived RBCs: progress, challenges and prospective solutions. Int J Mol Sci. 2021;22:9808. DOI: 10.3390/ijms22189808
79. Vazquez-Mellado MJ, Monjaras-Embriz V, Rocha-Zavaleta L. Erythropoietin, stem cell factor, and cancer cell migration. Vitam Horm. 2017;105:273–96. DOI: 10.1016/bs.vh.2017.02.008
80. Jacobsen RN, Nowlan B, Brunck ME, Barbier V, Winkler IG, Levesque J-P. Fms-like tyrosine kinase 3 (Flt3) ligand depletes erythroid island macrophages and blocks medullar erythropoiesis in the mouse. Exp Hematol. 2016;44:207–12.e204. DOI: 10.1016/j.exphem.2015.11.004
81. Valletta S, Thomas A, Meng Y, Ren X, Drissen R, Sengül H, et al. Micro-environmental sensing by bone marrow stroma identifies IL-6 and TGFβ1 as regulators of hematopoietic ageing. Nat Commun. 2020;11:4075. DOI: 10.1038/s41467-020-17942-7
82. Yumine A, Fraser ST, Sugiyama D. Regulation of the embryonic erythropoietic niche: a future perspective. Blood Res. 2017;52:10–17. DOI: 10.5045/br.2017.52.1.10
83. Li J, Cao C, Xiang Y, Hong Z, He D, Zhong H, et al. TLT2 suppresses Th1 response by promoting IL-6 production in monocyte through JAK/STAT3 signal pathway in tuberculosis. Front Immunol. 2020;11:2031. DOI: 10.3389/fimmu.2020.02031
84. Bari S, Seah KKH, Poon Z, Cheung AMS, Fan X, Ong S-Y, et al. Expansion and homing of umbilical cord blood hematopoietic stem and progenitor cells for clinical transplantation. Biol Blood Marrow Transplant. 2015;21:1008–19. DOI: 10.1016/j.bbmt.2014.12.022
85. Singh J, Chen ELY, Xing Y, Stefanski HE, Blazar BR, Zúñiga-Pflücker JC. Generation and function of progenitor T cells from StemRegenin-1–expanded CD34+ human hematopoietic progenitor cells. Blood Adv. 2019;3:2934–48. DOI: 10.1182/bloodadvances.2018026575
86. Panch SR, Szymanski J, Savani BN, Stroncek DF. Sources of hematopoietic stem and progenitor cells and methods to optimize yields for clinical cell therapy. Biol Blood Marrow Transplant. 2017;23:1241–49. DOI: 10.1016/j.bbmt.2017.05.003
87. Ben Nasr M, D'addio F, Malvandi AM, Faravelli S, Castillo-Leon E, Usuelli V, et al. Prostaglandin E2 stimulates the expansion of regulatory hematopoietic stem and progenitor cells in type 1Diabetes. Front Immunol. 2018;9. DOI: 10.3389/fimmu.2018.01387
88. Christopher AC, Venkatesan V, Karuppusamy KV, Srinivasan S, Babu P, Azhagiri MKK, et al. Preferential expansion of human CD34(+) CD133(+) CD90(+) hematopoietic stem cells enhances gene-modified cell frequency for gene therapy. Hum Gene Ther. 2022;33:188–201. DOI: 10.1089/hum.2021.089
89. Ren Y, Cui Y, Feng J, Tan Y, Ren F, Zhang Y, et al. Synergistic effect and molecular mechanism of PVA and UM171 in ex vivo expansion of primitive hematopoietic stem cells. J Cell Biochem. 2023;125:79–88. DOI: 10.1002/jcb.30505
90. Hinge A, Xu J, Javier J, Mose E, Kumar S, Kapur R, et al. p190-B RhoGAP and intracellular cytokine signals balance hematopoietic stem and progenitor cell self-renewal and differentiation. Nat Commun. 2017;8:14382. DOI: 10.1038/ncomms14382
91. Xiao X, Lai W, Xie H, Liu Y, Guo W, Liu Y, et al. Targeting JNK pathway promotes human hematopoietic stem cell expansion. Cell Discov. 2019;5:2. DOI: 10.1038/s41421-018-0072-8

How to cite this article: Zhuo Q, Zhang Z, Fang X. New insights into the mechanisms of red blood cell enucleation: From basics to clinical applications. eJHaem. 2024;5:1301–11. https://doi.org/10.1002/jha2.1051