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Microenvironmental dynamics in steady-state and stress erythropoiesis

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Abstract

Anemia is a condition marked by a shortage of red blood cells or hemoglobin, resulting in a diminished ability of the blood to carry oxygen. In response to anemia or hypoxia, the body activates a compensatory mechanism known as stress erythropoiesis. This crucial physiological process results in increased erythrocyte production, particularly in extramedullary sites such as the spleen and liver, to restore adequate oxygen levels. Unlike steady-state erythropoiesis, which primarily occurs in the bone marrow, stress erythropoiesis depends on distinct progenitor cells and signaling pathways within a specialized erythroid niche in adult spleen and liver. This niche provides essential support for the proliferation, differentiation, and maturation of erythroid progenitors during anemic stress. The dynamics within this niche under stress conditions involve complex interactions between progenitor and niche cells. These interactions are regulated by specific molecular signals that adapt to the body's physiological demands, ensuring an appropriate response to stress. This review explores the cellular and molecular mechanisms governing these processes, highlighting the extrinsic pathways and cellular interactions during stress erythropoiesis. In addition, it underscores the need for future research to translate findings from murine models into therapeutic strategies for treating anemia-related diseases.

Key Words: Anemia; Erythroblastic island; Microenvironment; Niche; Stress Erythropoiesis

1. INTRODUCTION

Steady-state erythropoiesis ensures the ongoing generation of red blood cells (RBCs), which is crucial for efficient oxygen supply and delivery to tissues. This well-coordinated process maintains erythroid homeostasis under normal conditions.[1] However, stress conditions can inhibit this steady production of RBCs, initiating an increase in erythroid output to compensate for the sudden reduction in erythrocyte levels. To address this need, an alternative pathway known as stress erythropoiesis is activated, which is an important physiological response to anemia or hypoxic stress.[2] Stress erythropoiesis that has been studied extensively in mice occurs extramedullary in the adult spleen and liver.[3]

Distinct from bone marrow (BM) steady-state erythropoiesis, stress erythropoiesis primarily occurs in extramedullary sites, including the adult spleen and liver. It relies on specialized progenitor cells and distinct intrinsic signaling pathways.[4] During stress, multiple cytokines and growth factors are upregulated to expand and differentiate stress erythroid progenitors (SEP). Furthermore, various transcription factors modulate the response of these progenitors to the hypoxic and inflammatory conditions of anemic stress. These pathways are tightly regulated to enable a rapid erythropoietic response, restoring RBC levels and oxygen delivery efficiently.

Extrinsically, erythroid cells require a specialized microenvironment, termed as the erythroblastic island (EBI), which serves as a niche within the BM or spleen where steady-state and stress erythropoiesis occur, respectively. The EBI consists of a central macrophage surrounded by developing erythroid cells at various terminal differentiation stages.[5–7] The development and signaling within the EBI microenvironment differ between stress and steady-state erythropoiesis, characterized by unique interactions between progenitors and niche cells.

In this review, we will delve into the complex dynamics of the erythroid niche and the extrinsic pathways regulating steady-state and stress erythropoiesis. Our aim is to illuminate the adaptive mechanisms from the erythroid niche perspectives that ensure an effective erythroid response under both homeostatic and stress conditions, highlighting potential therapeutic targets for disorders related to impaired erythropoiesis.

2. STEADY-STATE VERSUS STRESS ERYTHROPOIESIS

2.1. Steady-state erythropoiesis

Hematopoietic stem cells (HSCs), predominantly located in the BM, have a distinctive capacity for self-renewal and differentiation into various blood cell lineages, including erythroid cells. This differentiation begins with HSCs forming multipotent progenitor cells (MPPs), which then develop into common myeloid progenitors (CMPs). CMPs subsequently differentiate into megakaryocyte-erythroid progenitors (MEPs),[8] which under the influence of erythropoietin (EPO), give rise

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to erythroid progenitors such as burst-forming unit-erythroid (BFU-E) and colony-forming unit-erythroid (CFU-E).[9] These progenitors differentiate into multiple stages of erythroblasts, which subsequently lose their nuclei and organelles to become reticulocytes and erythrocytes, enter the bloodstream and facilitate oxygen transport.[10–12]

This tightly regulated differentiation process is influenced by intrinsic molecular and signaling pathways, along with the extrinsic cellular microenvironment that provides specific signaling molecules, ensuring proper erythroid development and function.

2.2. Stress erythropoiesis and intrinsic molecular regulation

Under stress conditions, the continuous production of erythrocytes may be disrupted or insufficient, initiating a compensatory increase in erythroid output,[2] termed as stress erythropoiesis. It is often triggered by conditions that increase the body's need for RBCs, due to blood loss, hemolysis, hypoxia, or anemia. In addition, infections or inflammatory conditions can alter the BM environment, leading to an elevated demand for erythropoiesis to compensate for these stresses.[13] Although stress erythropoiesis plays a crucial role in responding to anemic stress, it can result in pathological conditions such as abnormal hematopoiesis, inflammation, and myeloproliferative disorders when dysregulated. Prolonged activation of this process may cause long-term hematologic changes, including disruptions in stem cell behavior, which can contribute to aging-related diseases and other complications.[14,15]

Recent findings suggest that the bone morphogenetic protein 4 (BMP4)-dependent stress erythropoiesis pathway is triggered to rapidly generate new erythrocytes, helping to maintain homeostasis until steady-state erythropoiesis resumes.[2] This process depends on a population of erythroid-restricted, self-renewing, BMP4-dependent SEPs.[3] Initially identified during the recovery from acute anemia, the BMP4-dependent pathway has also been characterized in chronic anemia models.[16] This pathway is particularly active in the adult spleen and liver in murine models and is further regulated by additional signals such as Hedgehog, stem cell factor (SCF), and hypoxia.[3,17–19]

Several transcription factors play crucial roles in regulating stress erythropoiesis, working together to ensure a rapid and effective response to anemic stress. Hypoxia-inducible factor (HIF) is activated under low oxygen conditions and is essential for upregulating EPO production, which drives the expansion of SEPs.[20,21] GATA1, a key regulator of both steady-state and stress erythropoiesis, controls the expression of genes critical for hemoglobin production and erythrocyte development.[22] B-cell lymphoma/leukemia 11A (BCL11A) regulates fetal hemoglobin expression and maintains the balance between erythroid proliferation and differentiation during stress conditions.[23] Forkhead box O3 (FOXO3) is involved in regulating oxidative stress responses, ensuring the survival of erythroid progenitors during stress and promoting efficient erythropoiesis.[24] SRY-box transcription factor 6 (SOX6) contributes to stress erythropoiesis by facilitating the maturation of erythroid cells, enhancing their survival rate, and supporting the overall maturation process during anemic stress.[25] These transcription factors collectively regulate the proliferation, survival, and differentiation of erythroid progenitors during stress erythropoiesis, enabling the body to respond swiftly and effectively to anemic conditions (**Fig. 1**).

2.3. Experimental models to study stress erythropoiesis

To gain deeper insights into the mechanisms controlling stress erythropoiesis in humans and to develop novel anemia therapies, various experimental models have been established. A major challenge, however, is assessing how accurately these models represent mechanisms in humans, given the difficulties of direct human studies. While observational data from anemic patients and cultures of primary erythroid cells provide useful information, murine models have proven particularly useful in clarifying the molecular mechanisms underlying stress erythropoiesis.

In vitro cultures of murine BM cells, supplemented with factors like BMP4, growth differentiation factor 15 (GDF-15), SCF, and EPO, have successfully replicated the process, demonstrating the production of self-renewing SEPs.[26]

In vivo studies in mice, utilizing models such as erythroid short-term radioprotection (STR),[27] phenylhydrazine (PHZ)-induced acute hemolytic anemia, and serial bleeding-induced anemia,[2,28] have indeed been instrumental in shedding light on the intrinsic molecular changes within SEPs. These studies have also provided valuable insights into the cellular and molecular interactions between SEPs and their surrounding microenvironment. These models simulate the stress conditions that erythroid cells undergo during anemia, allowing for a detailed examination of the adaptive responses and regulatory mechanisms at play.

In addition, infection-based models and simulations of inflammatory anemia, using agents such as heat-killed *Brucella abortus* (HKBA), *Salmonella*, and the cecal ligation and puncture (CLP) model, have been used to investigate the mechanisms underlying stress erythropoiesis in response to inflammatory stimulations.[29–31]

However, translating findings from murine models to humans remains complex, partly due to the anatomical difference that complicates the direct application of murine findings to human physiology.[2] For instance, in mice, extramedullary erythropoiesis occurs more readily in the spleen, whereas in humans, this process is less pronounced. In addition, the cellular and molecular regulation of erythroid progenitors within the human spleen during anemic stress remains largely unexplored.[32] In addition, the molecular pathways and gene expression profiles that regulate stress erythropoiesis vary significantly between species, leading to potential discrepancies in the interpretation of experimental results. These differences underscore the need for careful consideration when extrapolating data from animal models to human conditions and highlight the importance of validating key findings derived from murine models in human cells or tissues whenever possible.

3. MICROENVIRONMENT IN STEADY-STATE ERYTHROPOIESIS

3.1. EBI macrophage functions

Central macrophages are vital for supporting erythroid progenitors by delivering crucial signals, nutrients, and structural support, to facilitate the proliferation, differentiation, and maturation of erythroblasts into functional RBCs.[5–7] The EBI macrophage niche plays a key role in maintaining erythroid homeostasis under both steady-state and stress conditions, adjusting to physiological demands such as increased erythropoiesis during anemia or hypoxia.

EBI macrophages are essential for erythroblast maturation, as they provide growth factors like insulin-like growth factor-1 (IGF-1) to promote erythroblast proliferation and differentiation, and supply iron necessary for hemoglobin synthesis.[33] Moreover, macrophages are essential to the enucleation process, where they engulf the nuclei expelled by newly formed reticulocytes. This critical function is highlighted by the significant enucleation defects observed in various knockout mouse models.[34] Notably, pyrenocyte is a structure of membrane-wrapped nuclei, which is expelled from erythroid cells during the enucleation process.[35] The degradation of pyrenocytes by EBI macrophages is mediated through the Mer tyrosine kinase (MerTK) pathway, which facilitates the attachment of pyrenocytes to EBI macrophages.[36] In addition, DNase 2α plays a role to digest the

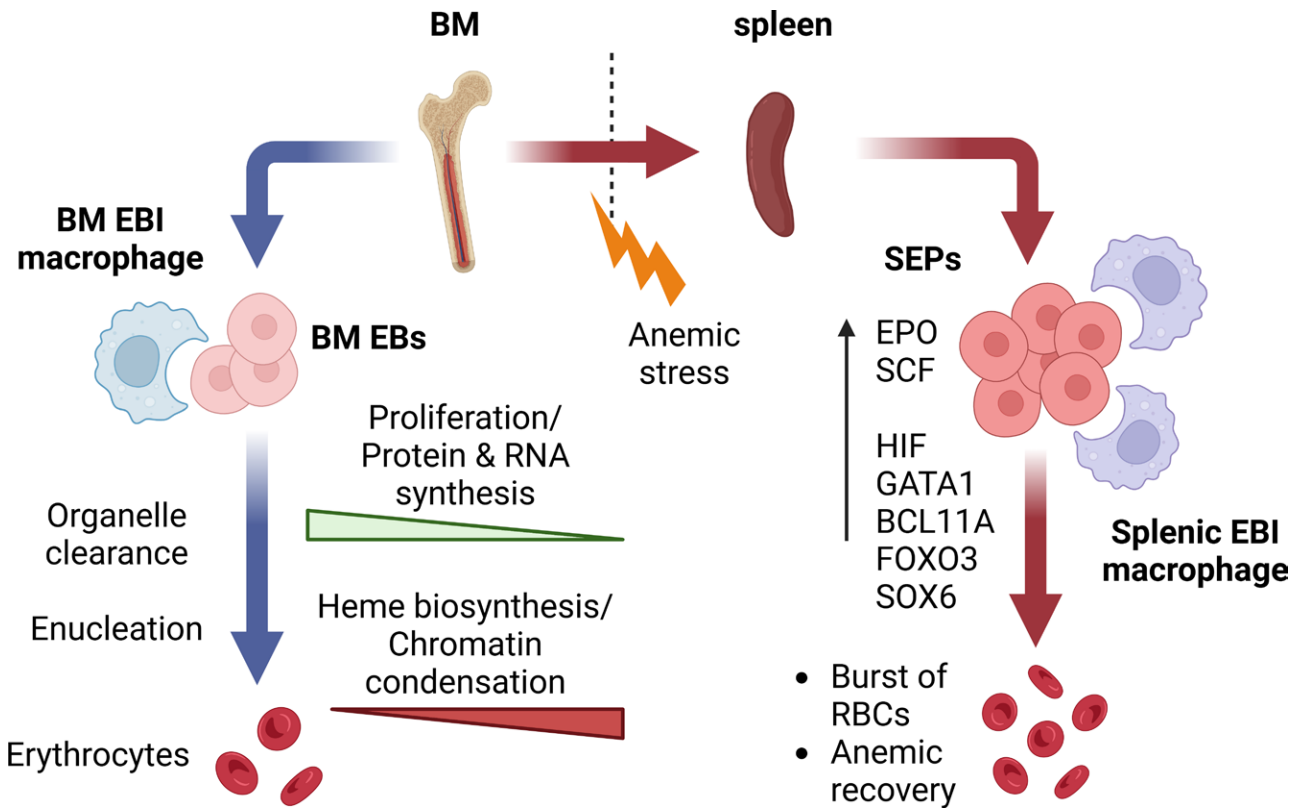

Figure 1. Overview of steady-state and stress erythropoiesis. In steady-state erythropoiesis, erythroid differentiation takes place in the BM. This process involves the progression through various stages of EB, culminating in enucleation within the EBI niche to form reticulocytes and RBCs. During these stages, heme biosynthesis, chromatin condensation, and organelle clearance are critical. In response to anemic stress, stress erythropoiesis is activated, predominantly in the spleen as an extramedullary site. This process involves the expansion of SEPs, driven by multiple signaling pathways, including EPO and SCF, and regulated by various transcription factors. Together, these elements induce a rapid burst of RBC production, facilitating erythroid recovery. BM = bone marrow, EB = erythroblasts, EBI = erythroblastic island, EPO = erythropoietin, RBC = red blood cells, SEPs = stress erythroid progenitors, SCF = stem cell factor. The illustration was created with BioRender.com.

nuclear DNA within the pyrenocyte.[37] These processes involve specific interactions between cell surface proteins on both macrophages and erythroid cells.

3.2. Interacting molecules on EBIs

The interaction between central EBI macrophages and erythroblasts is facilitated by specific adhesion molecules. Well-established EBI macrophage markers include αv integrin, vascular cell adhesion molecule 1 (VCAM-1; CD106), CD169, and F4/80, while erythroblasts display α4β1 integrin, intercellular adhesion molecule 4 (ICAM-4), and erythroblast macrophage protein (Emp).[6,38–41] This molecular interplay allows macrophages to effectively regulate the proliferation and differentiation of the interacting erythroblasts. The critical function of these adhesion molecules is evident from the significant enucleation defects observed in various knockout mouse models.[42,43]

In addition to these findings, recent studies have identified new markers on EBI macrophages and their corresponding receptors on erythroid cells, further elucidating their regulatory functions. For instance, the Ephrin and Eph receptor interaction, specifically ephrin-B2 on macrophages and EphB4 on erythroblasts, has been shown to play a critical role in erythroblast differentiation.[44]

Furthermore, CD163 has been recognized as an EBI macrophage marker, primarily expressed on EBI macrophages and interacts with erythroblasts. However, the interacting partner on erythroid cells has yet to be identified.[45] Interestingly, transcriptome analysis has shown that EBI macrophages express the EPO receptor in both mice and human fetal liver. This expression allows EPO to act on both erythroid cells and EBI macrophages at the same time. By increasing the expression of adhesion molecules in macrophages, EPO enhances EBI formation and influences efficient RBC production in an erythroid cell-extrinsic manner.[46]

4. MICROENVIRONMENT IN STRESS ERYTHROPOIESIS AND ANEMIA

4.1. Distinct EBI macrophage subtypes to regulate stress erythropoiesis

During stress conditions, the interaction between progenitors and macrophages within EBIs differs markedly from steady-state erythropoiesis. CD169+ EBI macrophages are especially important in stress erythropoiesis, where they play a pivotal role in the late stages of erythroid maturation and recovery from various anemic stressors. Their depletion leads to a reduced erythroblast count in the BM, resulting in a marked impairment of erythroid recovery.[38,47]

Another study highlights the multifaceted role of pro-inflammatory signals in regulating stress erythropoiesis. While inflammation is typically considered harmful to BM erythropoiesis due to its inhibitory effects on erythroid progenitor proliferation, it enhances the expansion and differentiation of SEPs in extramedullary sites, particularly the spleen. During PHZ-induced hemolytic anemia, there is a significant increase in splenic red pulp macrophages (RPMs), driven by monocyte recruitment through the Ccr2 signaling pathway. These monocytes migrate to the spleen and differentiate into mature EBI macrophages.

This process not only demonstrates the dynamic plasticity of the spleen's microenvironment but also underscores the crucial role of monocyte recruitment and differentiation to mediate stress erythropoiesis. These findings provide valuable insights into modulating the splenic response to anemic stress by targeting inflammatory signals and monocyte recruitment pathways.[48]

4.2. Bidirectional mitochondria transfer in EBI microenvironment

Interestingly, recent studies revealed the bidirectional transfer of mitochondria within EBIs between erythroid cells and their macrophage niche under various conditions. In steady-state erythropoiesis, mitochondria are cleared from erythroblasts primarily through autophagy. However, emerging findings highlight that mitochondrial transfer between cells provides additional support for the mitochondria clearance process. Specifically, early-stage erythroblasts transfer mitochondria to EBI macrophages through mechanisms such as tunneling nanotubes (TNTs).[49] This intercellular transfer of mitochondria facilitates their clearance which is necessary for erythroid maturation.

During acute anemic conditions, mitochondria transfer from EBI macrophages to early-stage erythroblasts occurs, enhancing erythroid recovery. Infusing EBI macrophages into anemic mice demonstrated this transfer, which was linked to improved erythroid recovery. Single-cell RNA sequencing revealed that erythroblasts acquiring exogenous mitochondria exhibited heightened metabolic activity, characterized by high CD47 expression. Blocking CD47 or its ligand Sirpα reduced mitochondria transfer and erythroid recovery, highlighting the necessity of the CD47–Sirpα interaction in this process. These findings emphasize the importance of mitochondria transfer in boosting erythroid recovery by altering erythroblast bioenergetics.[50] The bidirectional transfer of mitochondria between erythroblasts and macrophages plays a crucial role in maintaining erythroid homeostasis and adapting to physiological needs under both steady-state and stress conditions.

Collectively, these findings highlight the role of EBI macrophages as crucial regulators of erythroid recovery during stress and pathological erythropoiesis, suggesting that targeting these niche cells could present a promising therapeutic strategy for managing erythropoietic disorders.

5. MOLECULAR PATHWAYS AND SIGNALING DURING ANEMIC STRESS

5.1. GDF-15 regulatory pathway

GDF-15, a cytokine within the transforming growth factor beta (TGF-β) superfamily, plays a crucial role in immune tolerance and is significantly elevated in various stress conditions, often correlating with disease severity in cancer and other disorders. Under stress, macrophages produce high levels of GDF-15, influencing tissue fibrosis and angiogenesis. Given that macrophage-mediated inflammatory responses significantly impact the pathogenesis of many human diseases, GDF-15, with its potential anti-inflammatory properties, emerges as an important modulator of these pathological processes.[51]

Studies indicate that while GDF-15 may not be a crucial niche factor during steady-state erythropoiesis, it plays a vital regulatory role during stress erythropoiesis. GDF-15 orchestrates the microenvironment of erythroid progenitor cells under stress, aligning with their metabolic demands to support the response to anemic stress. It facilitates the proliferation of SEPs in the spleen, primarily via the expansion of monocyte-derived cells. In addition, GDF-15 upholds the expression of Bmp4 within the niche under hypoxic conditions and regulates the expression of metabolic enzymes in SEPs, thereby enhancing their proliferative capacity.[52]

Another study demonstrated that during recovery from bone marrow transplantation (BMT), splenic monocyte and macrophage populations were monitored, revealing that RPMs expressed high levels of F4/80, CD169, and Vcam1, while pre-RPMs from donor cells showed lower levels of these markers. In addition, an increase in Ccl2 mRNA expression was observed, along with a concurrent rise in the expression of Gdf15 and Bmp4, key regulators of stress erythropoiesis.[48]

5.2. SPI-C activation during stress erythropoiesis

SPI-C (Spi-C transcription factor) is selectively expressed in splenic RPMs, which are critical for the degradation of aged erythrocytes and the recycling of iron. RPMs development is activated by heme, a byproduct of erythrocyte breakdown. Heme has been shown to promote the proteasomal degradation of BTB domain and CNC homology 1 (BACH1), a transcriptional repressor of SPI-C, thereby relieving its inhibition and enhancing SPI-C expression.[53]

Notably, inflammation has been demonstrated to play a crucial role in initiating erythrophagocytosis by splenic macrophages, a process that is mediated through the activation of toll-like receptor (TLR) signaling pathways. This cascade of events leads to the activation of SPI-C, which is essential for orchestrating subsequent cellular responses. Once activated, SPI-C, in conjunction with continued TLR signaling, induces the expression of Gdf15 and Bmp4, which are key drivers of the expansion of SEPs within the spleen.[16] SPI-C triggers a vital response in splenic macrophages to compensate for inflammation-induced disruptions in steady-state erythropoiesis. This reflects a dynamic system that in response to inflammation, the body both boosts innate immune cell production and promotes stress erythropoiesis. This dual mechanism maintains erythroid homeostasis and preserves oxygen-carrying capacity during immune activation.

5.3. Cd81/C1q expressing EBI macrophages

Recent advancements in high-throughput sequencing, particularly single-cell multi-omics, have significantly advanced our understanding of EBI macrophages during anemic stress. These technologies enable the detailed dissection of cellular heterogeneity and the identification of specific transcriptional programs that govern the function of distinct types of macrophages within the erythroid niche. In one recent study, researchers used single-cell RNA sequencing on spleen tissue from mice subjected to various forms of anemic stress, revealing distinct transcriptional signatures in *Vcam1*+ macrophages. This analysis identified CD81 as a novel and crucial marker for central macrophages within EBIs, which are essential for mounting effective responses to anemic stress. The CD81+ macrophages exhibited resident macrophage gene expression profiles that distinguished them from other infiltrating macrophage subtypes, suggesting their specialized role in maintaining erythroid homeostasis under stress conditions.[28]

These findings were further substantiated by another study investigating macrophage subsets within EBIs in mice predisposed to systemic lupus erythematosus (SLE). This study identified 2 primary macrophage subsets within the EBIs: one subset expressing *Ly6c2* and *Ccr2*, and another subset expressing *Cd81* and complement component 1q (*C1q)*. Notably, C1q is primarily produced by monocyte lineages, such as macrophages, and is typically found at low levels in blood circulation under normal conditions.[54] In SLE, anti-C1q autoantibodies specifically target C1q bound to its natural ligands, enhancing Fc-receptor-mediated phagocytosis and potentially contributing to the pathology of autoimmune diseases.[55] Here, the *Cd81/C1q*-expressing macrophages displayed canonical markers of central macrophages and exhibited a pro-inflammatory profile.

These macrophages also downregulated genes critical for adhesion and nutrient provision to erythroid precursors, correlating with impaired erythropoiesis in the context of SLE. This suggests that the inflammatory environment in SLE disrupts the normal supportive functions of EBI macrophages, leading to defective erythropoiesis.[56]

Collectively, these studies provide invaluable insights into the intrinsic and extrinsic pathways that regulate erythroblast responses to stress. They also highlight the potential for therapeutic intervention in conditions where erythropoiesis is disrupted. By targeting specific macrophage subsets or modulating their transcriptional programs, it may be possible to restore effective erythropoiesis in diseases characterized by anemia and other hematologic abnormalities. Future research aimed at translating these findings into human therapies could lead to novel treatments for a range of disorders involving defective erythropoiesis, including chronic inflammatory conditions and autoimmune diseases such as SLE (**Fig. 2**).

6. ANEMIA AND NICHE REGULATIONS

Anemias that may rely on niche regulation are typically those associated with impaired or dysregulated hematopoiesis, where the BM or other hematopoietic niches fail to support normal RBC production. Examples of anemic disorders that may be regulated by niche interactions are listed.

6.1. Aplastic anemia (AA)

AA involves BM failure characterized by elevated immune responses, compromised HSC functions, and dysfunctional BM microenvironment.[57] Since AA is often linked to immune-mediated destruction of HSCs, the niche, including macrophages in the BM microenvironment, plays a crucial role in maintaining hematopoiesis, and dysregulation can contribute to the development of AA. Research indicates that AA patients exhibit higher occurrence of TNF-α–producing CD16+CD68+ macrophages in their BM compared to healthy controls. Moreover, patients with elevated levels of M2 macrophages tend to have better overall survival.[58,59]

6.2. Myelodysplastic syndromes (MDS)

MDS comprise a diverse group of hematological disorders characterized by ineffective hematopoiesis, resulting in anemia and other cytopenias. The BM niche, particularly the mesenchymal niche, plays a crucial role in disease initiation and progression. This niche, including stromal cells and various signaling

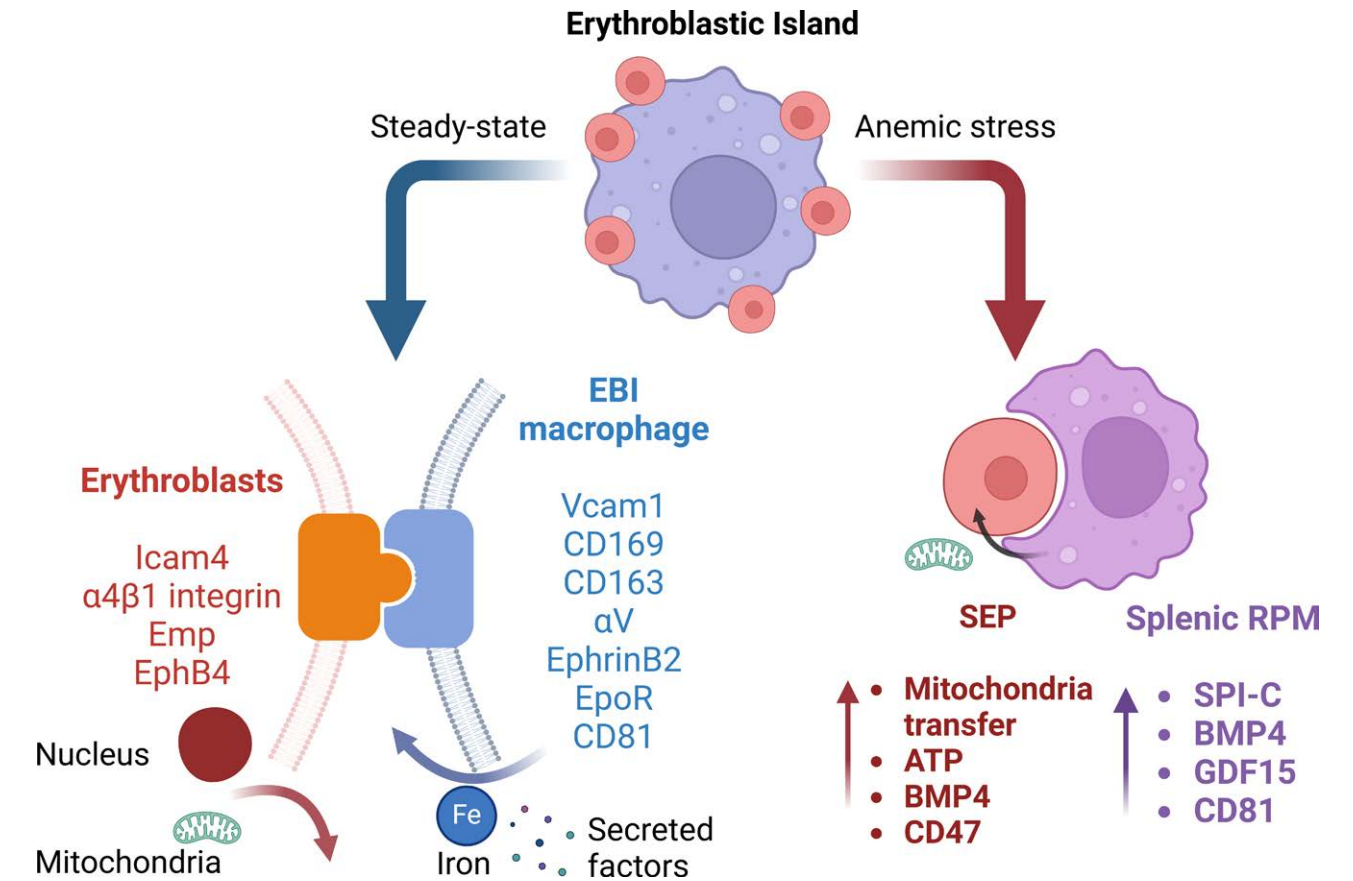

Figure 2. Intrinsic and extrinsic regulatory machineries of steady-state and stress erythropoiesis. Under steady-state conditions, erythroid differentiation within the bone marrow involves complex interactions between EB and EBI macrophages. Multiple binding partners, such as adhesion molecules and receptors, facilitate close physical interactions between these cells. This proximity enables efficient enucleation facilitated by EBI macrophages, as well as the transfer of mitochondria from erythroblasts to macrophages to promote terminal erythroid differentiation. In addition, the transport of iron and growth factors from macrophages to erythroblasts further supports effective erythroid maturation. During anemic stress, extrinsic signaling within the erythroid niche, particularly in splenic RPMs, is significantly altered. Key signaling pathways, including BMP4, transcription factor SPI-C, and GDF-15, are upregulated in these niche cells. These changes enhance the supportive environment for the expansion of SEPs. In addition, the transfer of mitochondria from niche cells to erythroblasts, facilitated by the upregulation of CD47, is augmented, providing essential energy (ie, ATP) and support for the rapid production of RBCs needed to counteract anemia. BMP4 = bone morphogenetic protein 4, EB = erythroblasts, EBI = erythroblastic island, GDF-15 = growth differentiation factor 15, RBC = red blood cells, RPMs = red pulp macrophages, SEPs = stress erythroid progenitors. The illustration was created with BioRender.com.

pathways, is implicated in the pathogenesis of MDS by influencing the proliferation and differentiation of hematopoietic cells.[60] Especially during aging, the BM microenvironment becomes increasingly inflammatory, causing mesenchymal stem cells (MSCs) to secrete pro-inflammatory factors, thereby elevating the risk of leukemogenesis.[61]

6.3. Diamond-Blackfan anemia (DBA)

DBA is a rare congenital anemia caused by defects in ribosomal proteins, resulting in defective erythropoiesis. Although most studies on DBA have traditionally focused on intrinsic erythroid defects, recent advancements in single-cell and spatial technologies have allowed for the investigation of non-erythroid and non-hematopoietic cells within the BM niches.[62] In DBA, triggered by ribosome-defective erythroid cells, the BM microenvironment including mesenchymal stromal cells becomes inflammatory and releases pro-inflammatory cytokines. This inflammatory state can influence the severity of the disease and the patient's response to treatment by producing reactive oxygen species (ROS), which leads to excessive oxidative stress and the activation of the DNA damage response.[63]

6.4. Thalassemia

Thalassemia is a collection of inherited blood disorders where the body is unable to produce enough hemoglobin, leading to anemia. A hallmark of thalassemia is ineffective erythropoiesis, often accompanied by iron overload, which can impair the BM microenvironment, particularly affecting the functions of MSCs and osteoblasts.[64] Therefore, targeting the BM microenvironment offers potential as a therapeutic strategy for treating thalassemia.

The interactions between erythroblasts or HSCs and their niche can impact the severity of anemia. In these conditions, therapeutic strategies targeting the hematopoietic niche, or its regulatory mechanisms may help improve erythropoiesis and alleviate anemia.

7. FUTURE DIRECTIONS

Despite these advancements in erythroid niche research under anemic conditions, several intriguing questions remain unanswered. How do specific signaling molecules and pathways orchestrate the recruitment and differentiation of erythroid progenitors during anemia? Other than EBI macrophages, are other types of immune cells involved in the mediation of erythroid recovery from stress? What are the precise mechanisms behind the bidirectional transfer of mitochondria between erythroblasts and macrophages, and how do these exchanges influence erythroid recovery? Unraveling these mysteries will require imaginative experimental designs and interdisciplinary collaborations, blending insights from cell biology, immunology, and bioinformatics.

The future of erythroid niche research under anemic conditions is set to be an exciting frontier, fueled by cutting-edge technologies and innovative methodologies. Emerging technologies such as single-cell and spatial multi-omics, along with advanced imaging methods, are expanding our understanding of the complicated cellular interactions and molecular pathways within the erythroid niche. These technologies enable us to explore the interactions between erythroblasts and novel niche cells, including immune cells beyond EBI macrophages, mapping the emerging interacting molecules involved and deciphering the mechanisms underlying these interactions.

Despite significant progress, several critical questions remain unanswered, particularly regarding how the erythroid niche, especially macrophages, facilitates erythroblast enucleation through extrinsic mechanisms beyond the engulfment and degradation of nuclei in pyrenocytes.[36] Macrophages may influence the composition of the extracellular matrix (ECM) and the expression of adhesion molecules in the erythroid niche, affecting erythroblast interactions with their environment and promoting enucleation. The involvement of integrins and other adhesion molecules could be pivotal in modulating cytoskeletal dynamics during this process. In addition, erythroid niche secretes cytokines and growth factors, such as IGF-1, interleukin-18 (IL-18), and vascular endothelial growth factor B (VEGF-B), which may activate signaling pathways in erythroblasts,[46] leading to chromatin condensation and subsequent enucleation. Investigating these signaling pathways could reveal how they work in concert with intrinsic factors within erythroblasts to facilitate nucleus extrusion. Super-resolution imaging, including fluorescence electron microscopy techniques, offers the potential to uncover these mechanisms, providing unprecedented resolution to map the dynamic landscape of EBI macrophages and erythroblasts.

Given that the niche microenvironment plays a critical role in the regulation and pathogenesis of various anemic disorders, including AA, MDS, DBA, and thalassemia, targeting niche-interacting molecules with hematopoietic cells or modulating the pro-inflammatory cytokine signaling pathways could offer significant therapeutic potential. Moreover, the potential for personalized medicine in this field is largely untapped. By leveraging genomics and proteomics, we can envision a future where treatments for erythropoietic disorders are tailored to the unique molecular and genetic profiles of individual anemic patients. This personalized approach promises more effective and targeted therapies, minimizing side effects while optimizing therapeutic outcomes. In the quest to modulate the erythroid niche for therapeutic purposes, several promising avenues emerge. For instance, the therapeutic manipulation of interacting molecules and pro-inflammatory pathways in niche cells could enhance erythroid recovery and revolutionize anemia treatment. In addition, investigating the role of epigenetic regulation and transcription factors in EBI macrophages may uncover novel strategies to hinder red cell pathogenesis or promote healthy RBC production.

Ultimately, the journey to fully understand and harness the erythroid niche will be a blend of meticulous science and imaginative exploration. As we venture into this promising future, the integration of emerging technologies, the resolution of long-standing questions, and the realization of personalized medicine will transform our approach to managing anemia and related disorders.

8. CONCLUSION

Stress erythropoiesis is a vital compensatory mechanism that boosts RBC production during anemic conditions and is well-coordinated by intrinsic and extrinsic mechanisms, including specialized progenitor cells, unique microenvironments, and distinct signaling pathways that adapt dynamically to stress. Although murine models have significantly advanced our understanding of these processes, translating these findings to human medicine is challenging due to species-specific differences. Future research aims to address these challenges by elucidating the molecular mechanisms within the human erythroid niche, utilizing advanced technologies such as cutting-edge imaging techniques and single-cell and spatial multi-omics. These efforts are geared toward personalized medicine, focusing on individual molecular profiles to develop more effective and tailored therapies for anemia and related disorders such as AA, MDS, DBA, and thalassemia, thereby enhancing clinical outcomes and offering new hope for patients.

AUTHOR CONTRIBUTIONS

C.Y. conducted literature research and drafted the manuscript. T.S. edited and finalized the manuscript.

REFERENCES

[1] Palis J. Primitive and definitive erythropoiesis in mammals. *Front Physiol* 2014;5:3.
[2] Paulson RF, Hariharan S, Little JA. Stress erythropoiesis: definitions and models for its study. *Exp Hematol* 2020;89:43–54.e2.
[3] Paulson RF, Shi L, Wu DC. Stress erythropoiesis: new signals and new stress progenitor cells. *Curr Opin Hematol* 2011;18(3):139–145.
[4] Bennett LF, Liao C, Paulson RF. Stress erythropoiesis model systems. *Methods Mol Biol (Clifton, NJ)*. 2018;1698:91–102.
[5] Li W, Guo R, Song Y, Jiang Z. Erythroblastic island macrophages shape normal erythropoiesis and drive associated disorders in erythroid hematopoietic diseases. *Front Cell Dev Biol* 2020;8:613885.
[6] Li W, Wang Y, Chen L, An X. Erythroblast island macrophages: recent discovery and future perspectives. *Blood Sci* 2019;1(1):61–64.
[7] Manwani D, Bieker JJ. The erythroblastic island. *Curr Top Dev Biol* 2008;82:23–53.
[8] Seita J, Weissman IL. Hematopoietic stem cell: self-renewal versus differentiation. *Wiley Interdiscip Rev Syst Biol Med* 2010;2(6):640–653.
[9] Bhoopalan SV, Huang LJ, Weiss MJ. Erythropoietin regulation of red blood cell production: from bench to bedside and back. *F1000Research* 2020;9:F1000 Faculty Rev–F1000 Faculty1153.
[10] 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(24):6258–6268.
[11] Schippel N, Sharma S. Dynamics of human hematopoietic stem and progenitor cell differentiation to the erythroid lineage. *Exp Hematol* 2023;123:1–17.
[12] Moras M, Lefevre SD, Ostuni MA. From erythroblasts to mature red blood cells: organelle clearance in mammals. Mini Review. *Front Physiol* 2017;8:1076.
[13] Paulson RF, Ruan B, Hao S, Chen Y. Stress erythropoiesis is a key inflammatory response. *Cells* 2020;9(3):634.
[14] Barisas DAG, Choi K. Extramedullary hematopoiesis in cancer. *Exp Mol Med* 2024;56(3):549–558.
[15] Kim A, Nemeth E. New insights into iron regulation and erythropoiesis. *Curr Opin Hematol* 2015;22(3):199–205.
[16] Bennett LF, Liao C, Quickel MD, et al. Inflammation induces stress erythropoiesis through heme-dependent activation of SPI-C. *Sci Signaling* 2019;12(598):eaap7336.
[17] Lenox LE, Perry JM, Paulson RF. BMP4 and Madh5 regulate the erythroid response to acute anemia. *Blood* 2005;105(7):2741–2748.
[18] Perry JM, Harandi OF, Paulson RF. BMP4, SCF, and hypoxia cooperatively regulate the expansion of murine stress erythroid progenitors. *Blood* 2007;109(10):4494–4502.
[19] Perry JM, Harandi OF, Porayette P, Hegde S, Kannan AK, Paulson RF. Maintenance of the BMP4-dependent stress erythropoiesis pathway in the murine spleen requires hedgehog signaling. *Blood* 2009;113(4):911–918.
[20] Flygare J, Estrada VR, Shin C, Gupta S, Lodish H. Stress erythropoiesis: HIF-1 alpha synergizes with glucocorticoids to induce BFU-E progenitor self-renewal. *Blood* 2010;116(21):814–814.
[21] Haase VH. Regulation of erythropoiesis by hypoxia-inducible factors. *Blood Rev* 2013;27(1):41–53.
[22] Gutiérrez L, Tsukamoto S, Suzuki M, et al. Ablation of Gata1 in adult mice results in aplastic crisis, revealing its essential role in steady-state and stress erythropoiesis. *Blood* 2008;111(8):4375–4385.
[23] Demirci S, Zeng J, Wu Y, et al. BCL11A enhancer–edited hematopoietic stem cells persist in rhesus monkeys without toxicity. *J Clin Invest* 2020;130(12):6677–6687.
[24] Marinkovic D, Zhang X, Yalcin S, et al. Foxo3 is required for the regulation of oxidative stress in erythropoiesis. *J Clin Invest* 2007;117(8):2133–2144.
[25] Dumitriu B, Bhattaram P, Dy P, et al. Sox6 is necessary for efficient erythropoiesis in adult mice under physiological and anemia-induced stress conditions. *PLoS One* 2010;5(8):e12088.
[26] Xiang J, Wu DC, Chen Y, Paulson RF. In vitro culture of stress erythroid progenitors identifies distinct progenitor populations and analogous human progenitors. *Blood* 2015;125(11):1803–1812.
[27] Harandi OF, Hedge S, Wu DC, McKeone D, Paulson RF. Murine erythroid short-term radioprotection requires a BMP4-dependent, self-renewing population of stress erythroid progenitors. *J Clin Invest* 2010;120(12):4507–4519.
[28] Yang C, Yokomori R, Chua LH, et al. Deciphering the regulatory landscape of murine splenic response to anemic stress at single-cell resolution. *Blood Adv* 2024;8(7):1651–1666.
[29] Gardenghi S, Renaud TM, Meloni A, et al. Distinct roles for hepcidin and interleukin-6 in the recovery from anemia in mice injected with heat-killed Brucella abortus. *Blood* 2014;123(8):1137–1145.
[30] Mishra SK, Choudhury S. Experimental protocol for cecal ligation and puncture model of polymicrobial sepsis and assessment of vascular functions in mice. *Methods Mol Biol* 2018;1717:161–187.
[31] Jackson A, Nanton MR, O'Donnell H, Akue AD, McSorley SJ. Innate immune activation during Salmonella infection initiates extramedullary erythropoiesis and splenomegaly. *J Immunol* 2010;185(10):6198–6204.
[32] Mende N, Bastos HP, Santoro A, et al. Unique molecular and functional features of extramedullary hematopoietic stem and progenitor cell reservoirs in humans. *Blood* 2022;139(23):3387–3401.
[33] Lévesque J-P, Summers KM, Bisht K, Millard SM, Winkler IG, Pettit AR. Macrophages form erythropoietic niches and regulate iron homeostasis to adapt erythropoiesis in response to infections and inflammation. *Exp Hematol* 2021;103:1–14.
[34] Keerthivasan G, Wickrema A, Crispino JD. Erythroblast enucleation. *Stem Cells Int* 2011;2011:139851.
[35] Toda S, Nishi C, Yanagihashi Y, Segawa K, Nagata S. Clearance of apoptotic cells and pyrenocytes. *Curr Top Dev Biol* 2015;114:267–295.
[36] Toda S, Segawa K, Nagata S. MerTK-mediated engulfment of pyrenocytes by central macrophages in erythroblastic islands. *Blood* 2014;123(25):3963–3971.
[37] Kawane K, Fukuyama H, Kondoh G, et al. Requirement of DNase II for definitive erythropoiesis in the mouse fetal liver. *Science* 2001;292(5521):1546–1549.
[38] Chow A, Huggins M, Ahmed J, et al. CD169^{+} macrophages provide a niche promoting erythropoiesis under homeostasis and stress. *Nat Med* 2013;19(4):429–436.
[39] Mankelow TJ, Spring FA, Parsons SF, et al. Identification of critical amino-acid residues on the erythroid intercellular adhesion molecule-4 (ICAM-4) mediating adhesion to alpha V integrins. *Blood* 2004;103(4):1503–1508.
[40] Sadahira Y, Yoshino T, Monobe Y. Very late activation antigen 4-vascular cell adhesion molecule 1 interaction is involved in the formation of erythroblastic islands. *J Exp Med* 1995;181(1):411–415.
[41] Soni S, Bala S, Hanspal M. Requirement for erythroblast-macrophage protein (Emp) in definitive erythropoiesis. *Blood Cells Mol Dis* 2008;41(2):141–147.
[42] Lee G, Lo A, Short SA, et al. Targeted gene deletion demonstrates that the cell adhesion molecule ICAM-4 is critical for erythroblastic island formation. *Blood* 2006;108(6):2064–2071.
[43] Soni S, Bala S, Gwynn B, Sahr KE, Peters LL, Hanspal M. Absence of erythroblast macrophage protein (Emp) leads to failure of erythroblast nuclear extrusion. *J Biol Chem* 2006;281(29):20181–20189.
[44] Lea AH-ON, Charlotte ES, Stephen JC, Sonam G, Catherine DN, Ashley MT. Ephrin/Eph receptor interaction facilitates macrophage recognition of differentiating human erythroblasts. *Haematologica* 2020;105(4):914–924.
[45] Fabriek BO, Polfliet MMJ, Vloet RPM, et al. The macrophage CD163 surface glycoprotein is an erythroblast adhesion receptor. *Blood* 2007;109(12):5223–5229.
[46] Li W, Wang Y, Zhao H, et al. Identification and transcriptome analysis of erythroblastic island macrophages. *Blood* 2019;134(5):480–491.
[47] Ramos P, Casu C, Gardenghi S, et al. Macrophages support pathological erythropoiesis in polycythemia vera and β-thalassemia. *Nat Med* 2013;19(4):437–445.
[48] Liao C, Prabhu KS, Paulson RF. Monocyte-derived macrophages expand the murine stress erythropoietic niche during the recovery from anemia. *Blood* 2018;132(24):2580–2593.
[49] Yang C, Endoh M, Tan DQ, et al. Mitochondria transfer from early stages of erythroblasts to their macrophage niche via tunnelling nanotubes. *Br J Haematol* 2021;193(6):1260–1274.
[50] Yang C, Yokomori R, Chua LH, et al. Mitochondria transfer mediates stress erythropoiesis by altering the bioenergetic profiles of early erythroblasts through CD47. *J Exp Med* 2022;219(12):e20220685.
[51] Dai C, Zhang H, Zheng Z, et al. Identification of a distinct cluster of GDF15(high) macrophages induced by in vitro differentiation exhibiting anti-inflammatory activities. *Front Immunol* 2024;15:1309739.
[52] Hao S, Xiang J, Wu D-C, et al. Gdf15 regulates murine stress erythroid progenitor proliferation and the development of the stress erythropoiesis niche. *Blood Adv* 2019;3(14):2205–2217.
[53] Haldar M, Kohyama M, So AY, et al. Heme-mediated SPI-C induction promotes monocyte differentiation into iron-recycling macrophages. *Cell* 2014;156(6):1223–1234.

[54] Ghebrehiwet B, Hosszu KH, Peerschke EI. C1q as an autocrine and paracrine regulator of cellular functions. *Mol Immunol* 2017;84: 26–33.
[55] Dijkstra DJ, van de Bovenkamp FS, Abendstein L, et al. Human anti-C1q autoantibodies bind specifically to solid-phase C1q and enhance phagocytosis but not complement activation. *Proc Natl Acad Sci USA* 2023;120(50):e2310666120.
[56] Maria NI, Papoin J, Raparia C, et al. Human TLR8 induces inflammatory bone marrow erythromyeloblastic islands and anemia in SLE-prone mice. *Life Sci Alliance* 2023;6(10):e202302241.
[57] Seyfried AN, McCabe A, Smith JNP, Calvi LM, MacNamara KC. CCR5 maintains macrophages in the bone marrow and drives hematopoietic failure in a mouse model of severe aplastic anemia. *Leukemia* 2021;35(11):3139–3151.
[58] Sun W, Wu Z, Lin Z, et al. Macrophage TNF-α licenses donor T cells in murine bone marrow failure and can be implicated in human aplastic anemia. *Blood* 2018;132(26):2730–2743.
[59] Vaht K, Brenner J, Ednersson SB, Ljungman P, Brune M, Andersson PO. Bone marrow expression of CD68/CD163 macrophages, IL-17 and FOXP3 cells in aplastic anemia and their relation to prognosis. *Eur J Haematol* 2023;110(3):313–321.
[60] Pronk E, Raaijmakers M. The mesenchymal niche in MDS. *Blood* 2019;133(10):1031–1038.
[61] Friedrich C, Kosmider O. The mesenchymal niche in myelodysplastic syndromes. *Diagnostics (Basel)* 2022;12(7):1639.
[62] Iskander D, Karadimitris A, Roberts I. Harnessing single-cell technologies in the search for new therapies for Diamond–Blackfan anemia syndrome. *Exp Hematol* 2024;135:104235.
[63] Kapralova K, Jahoda O, Koralkova P, et al. Oxidative DNA damage, inflammatory signature, and altered erythrocytes properties in Diamond-Blackfan anemia. *Int J Mol Sci* 2020;21(24):9652.
[64] Li Z, Yao X, Zhang J, Yang J, Ni J, Wang Y. Exploring the bone marrow micro environment in thalassemia patients: potential therapeutic alternatives. *Front Immunol* 2024;15:1403458.