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Erythroferrone in focus: emerging perspectives in iron metabolism and hematopathologies

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Abstract

Beyond its core role in iron metabolism, erythroferrone (ERFE) has emerged as a key player with far-reaching implications in various hematologic disorders. Its regulatory effect on hepcidin underlines its significance in conditions characterized by disrupted iron homeostasis. In β-thalassemia and myelodysplastic syndromes, its dysregulation intricately contributes to the clinical challenges of anemia and iron overload which highlights its potential as a therapeutic target. In anemia of chronic disease and iron deficiency anemia, ERFE presents a unique profile. In chronic kidney disease (CKD), the intricate interplay between ERFE, erythropoietin, and hepcidin undergoes dysregulation, contributing to the complex iron imbalance characteristic of this condition. Recent research suggests that ERFE plays a multifaceted role in restoring iron balance in CKD, beyond simply suppressing hepcidin production. The potential to modulate ERFE activity offers a novel approach to treating a spectrum of disorders associated with iron dysregulation. As our understanding of ERFE continues to evolve, it is poised to become a key focus in the development of targeted treatments, making it an exciting and dynamic area of ongoing research. Modulating ERFE activity presents a groundbreaking approach to treat iron dysregulation in conditions like iron deficiency anemia, thalassemia, and hemochromatosis. As new research unveils its intricate roles, ERFE has rapidly emerged as a key target for developing targeted therapies like ERFE agonists and antagonists. With promising studies underway, this dynamic field holds immense potential to improve patient outcomes, reduce complications, and offer personalized treatment options in hematology research. This comprehensive overview of ERFE's role across various conditions underscores its pivotal function in iron metabolism and associated pathologies.

Key Words: Erythroferrone; Hepcidin; Iron; Iron metabolism

1. INTRODUCTION

Erythroferrone (ERFE), discovered in 2014,[1] has revolutionized our understanding of iron regulation by serving as a key communication channel between erythroid progenitors in the bone marrow and hepatocytes. Synthesized in response to erythropoietin (EPO) stimulation, this crucial hormone predominantly targets hepatocytes, modulating hepcidin levels, the master regulator of iron homeostasis.[2] Understanding ERFE is crucial for unraveling the complexities of iron metabolism in both health and disease. The discovery of ERFE has expanded our understanding of iron metabolism and opened new therapeutic avenues for iron-related disorders.

This review article delves into the multifaceted roles of ERFE, exploring its historical discovery, intricate synthesis and regulation, and its dynamic interaction with hepcidin. We also explore the diverse roles of ERFE in both health and disease, culminating in an analysis of its potential as a therapeutic target, particularly in conditions like thalassemia, among others. To ensure a comprehensive and up-to-date analysis of ERFE's role in iron metabolism and hematologic disorders, a diversified search strategy was employed. We conducted thorough and systematic searches across multiple scientific databases and digital libraries, including PubMed, Google Scholar, and Web of Science. Our search utilized specific keywords like "erythroferrone," "iron metabolism," and "hematologic disorders." Additionally, we accessed specialized databases like Scopus and MEDLINE for broader coverage and consulted official websites of notable hematological and medical associations for expert opinions and guidelines. This multi-dimensional approach ensured a robust and inclusive analysis of the current research landscape.

1.1. Historical perspective

The journey toward ERFE's discovery began with intriguing observations indicating an erythroid-derived factor influencing iron homeostasis.[3] Researchers noted that the body managed iron levels dynamically, increasing absorption and mobilization during anemia or hypoxia. The breakthrough in 2014 provided a molecular link between erythropoiesis and hepcidin regulation, revealing that EPO-induced ERFE production in erythroblasts acts directly to suppress hepcidin production.[1] This discovery filled a crucial gap in our understanding of iron homeostasis.

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The early inklings of ERFE's existence emerged from studies on anemia of inflammation (AI), where it was observed that inflammatory cytokines like interleukin (IL)-6 could suppress erythropoiesis and induce hepcidin expression.[4] Researchers hypothesized that erythroid progenitors might produce a factor that could suppress hepcidin, thereby increasing iron availability for erythropoiesis. This hypothesis was confirmed with ERFE's identification in 2014.[1]

1.2. Structural characteristics

ERFE, also known as CTRP15 or myonectin, is encoded by the *FAM132B* gene in humans.[5,6] Initially, it was discovered as a secreted molecule belonging to the C1q/TNF-related protein (CTRP) family.[7] While primarily expressed in erythropoietic tissues and the spleen, which serve as the primary sites for erythropoiesis and stress erythropoiesis in mice, as evidenced by increased Erfe mRNA levels following hemorrhage or EPO stimulation,[1,8] ERFE has also been detected in various non-erythropoietic sites including the liver, heart, adipose tissues, and muscles.[9–12] Interestingly, ERFE expression in these non-erythropoietic tissues remains relatively low compared to that in the bone marrow and spleen. This suggests that ERFE might play additional roles beyond its initial discovery in iron regulation.

Structurally, ERFE resembles other CTRP family members, sharing a common architecture that includes 4 distinct domains: an N-terminal "tag" facilitating its release from cells, a collagenous linker connecting 2 larger domains and potentially allowing the protein to interact with itself or other molecules, a variable domain unique to ERFE, responsible for its specific function and a C-terminal structure resembling components of the immune system, hinting at potential immune-related roles. The full-length human ERFE protein consists of 354 amino acids with a predicted mass of 37.3 kDa.[13] Post-translational modifications, including N-glycosylation of an asparagine residue and co-translational removal of the signal peptide, contribute to the mature human ERFE protein exhibiting an apparent molecular weight ranging from 35 to 40 kDa.[14] The globular C-terminal domain is expected to fold into the highly structured TNF/C1q head, while the N-terminus exhibits an extended and flexible secondary structure. Moreover, a short proline-rich collagenous linker connects the larger domains and likely facilitates protein multimerization, akin to the native forms of adiponectin, albeit lacking the lysines involved in higher-order multimer assembly.[15,16] ERFE also contains 2 predicted PCSK3/furin recognition sites, suggesting the existence of multiple cleaved isoforms following protein processing.[13] Figure 1 shows hypothetical structural model of ERFE.

1.3. Synthesis and regulation

The release of EPO from renal parenchymal cells acts as a pivotal hormone in erythropoiesis, not only stimulating the genesis of nascent erythrocytes but also augmenting the synthesis of ERFE within the erythroblasts residing in the bone marrow. This basal erythropoiesis operates at a relatively steady cadence within the medullary cavity, diligently replenishing senescent and compromised red cells, whose iron constituents are predominantly reclaimed through the phagocytic activities of splenic and hepatic macrophages.

In the physiological context of anemia or systemic hypoxia, a decrease in oxygen delivery to the tissues occurs. This condition, recognized as cellular hypoxia, triggers a cascade of events within the kidneys. Specifically, specialized cells called "interstitial fibroblasts" located in high-energy-demand areas of the renal medulla sense the oxygen reduction. These cells act as sentinels, orchestrating a coordinated response. They activate a key molecule known as hypoxia-inducible factor-2 (HIF-2), which subsequently induces the synthesis of EPO.[17,18] The marked increase in plasma EPO levels not only significantly enhances the survival of erythroid progenitors but also amplifies the proliferation of erythroblasts, thereby further stimulating these cells to synthesize and secrete ERFE.

2. ERFE AND HEPCIDIN: REGULATORY RELATIONSHIP

The primary function of ERFE as a negative regulator of hepcidin is central to iron homeostasis. This ERFE-hepcidin axis ensures a balance between iron absorption, mobilization, and storage, adapting to the body's varying demands for erythropoiesis.

Hepcidin is a small peptide primarily synthesized by hepatocytes. It plays an indispensable role in maintaining systemic iron homeostasis.[19,20] Hepcidin regulates serum iron levels by binding to ferroportin (FPN), the sole known iron export transmembrane protein found in red cells, macrophages, enterocytes, and hepatocytes.[21] Binding of hepcidin to FPN diminishes cellular iron export through two mechanisms. Firstly, hepcidin induces a conformational change in FPN, initiating its ubiquitination, and subsequent degradation. Secondly, the binding of hepcidin to FPN occludes iron transport. At the organismal level, decreased FPN activity results in reduced iron absorption and diminished mobilization from iron stores.[8,20] In healthy individuals, hepcidin homeostasis is closely tied to the iron levels of the body, including both plasma and the liver iron content. An excess of iron prompts a feedback mechanism to modulate iron levels by upregulating hepcidin concentration, while erythroid hyperplasia substantially reduces hepcidin levels to ensure an adequate iron supply for erythropoiesis.[6] Moreover, a significant increase in hepcidin levels has been observed during episodes of acute or chronic inflammation, potentially contributing to anemia of chronic disease(s) (ACD).[22] Low levels of hepcidin stabilize FPN at cellular membrane, promoting dietary iron absorption in the duodenum and enhancing iron release from macrophages following erythrophagocytosis, facilitating iron mobilization from hepatocytes.[23] Hepcidin levels remain suppressed in conditions associated with accelerated erythropoiesis, such as anemia due to bleeding, hemolysis, iron deficiency, and ineffective erythropoiesis.[24] This hepcidin suppression leads to iron overload in β-thalassemia.[9]

ERFE exerts an inhibitory effect on hepatic hepcidin production by modulating the bone morphogenetic protein (BMP)/

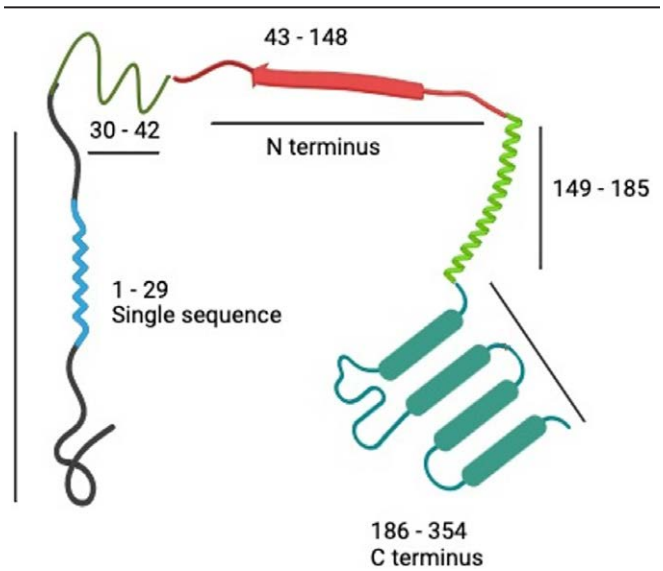

Figure 1. Structural model of erythroferrone.

SMAD (SMAD stands for "Sma" [from the Caenorhabditis elegans protein Sma] and "Mad" [from the Drosophila protein Mothers against decapentaplegic, or Mad]) signaling pathway within hepatocytes.[25,26] Specifically, ERFE inhibits the hepcidin-inducing effects of BMP5, BMP6, and BMP7, while leaving other BMPs such as BMP2, BMP4, BMP9, or activin B unaffected. This selectivity arises from the ability of ERFE to decrease the phosphorylation of SMAD1, SMAD5, and SMAD8, key signaling molecules downstream of these particular BMPs, thereby preventing the expression of genes normally activated by BMPs.[25,27] Phosphorylated SMAD1/5/8 forms a complex with SMAD4. This complex translocates into the nucleus, where it binds to specific DNA sequences in the promoter region of the hepcidin (*HAMP*) gene. The SMAD complex promotes the transcription of the *HAMP* gene.[2,8,25]

BMP5 mutations in mice recapitulate hepcidin deficiency, altered dietary iron response, and increased iron overload. ERFE directly binds BMP5, inhibiting its hepcidin-inducing activity, particularly under diminished BMP6 conditions, revealing a novel regulatory mechanism for iron hemostasis.[27] ERFE-mediated hepcidin reduction leads to FPN upregulation in iron-exporting cells, for example, macrophages and enterocytes, facilitating increased iron availability in plasma. This process is further augmented by enhanced dietary iron absorption via DMT1 and mobilization of ferritin-bound iron from macrophages and hepatocytes, ultimately fueling heme and hemoglobin synthesis for the new red cells (**Fig. 2, A**).[8] The role of ERFE is particularly pronounced in the early phases of response to erythropoietic stimuli. This is exemplified in ERFE knockout mice, which exhibits a marked delay in hepcidin suppression following acute hemorrhage or exogenous EPO administration, leading to a protracted recuperation period from anemia compared to their wild-type counterparts.[1] ERFE production increases in response to anemia through a dual mechanism: EPO-driven expansion of erythroid precursors and enhanced ERFE expression per cell. Notably, β-thalassemia major exhibits significant expansion of erythroid precursor population, but these cells fail to mature. Despite their inability to differentiate, these aberrant erythroid precursors secrete elevated levels of ERFE, leading to chronic hepcidin suppression and consequent iron overload, suggesting a potential mechanism for this pathological feature.[13,15]

When there is sufficient iron available and erythropoiesis is proceeding normally without additional demand for red cell production, the synthesis of ERFE is downregulated. This downregulation is likely mediated through the suppression of EPO production as a result of adequate oxygenation of tissues and the absence of erythropoietic stress. Additionally, the presence of adequate iron levels leads to the normalization of hepcidin levels, further reducing the need for ERFE-mediated suppression of hepcidin. This interplay ensures that ERFE synthesis is closely aligned with the body's erythropoietic activity and iron needs, preventing excessive iron mobilization and maintaining iron homeostasis.

3. PATHOPHYSIOLOGICAL IMPLICATIONS OF ERFE

In healthy individuals, ERFE ensures a fine-tuned balance between iron requirements and availability. It is particularly crucial in conditions associated with increased iron demand, such as pregnancy or growth phases. Disruptions in this regulatory pathway can lead to iron overload or deficiency, underscoring the need for a deeper understanding of ERFE's role in various dysregulated iron disorders.

3.1. β-Thalassemia

Thalassemia represents a heterogeneous array of hemoglobinopathies, predominantly inherited as autosomal-recessive disorder. It is notably characterized by diminished or lack of synthesis of either α or β globin chains of hemoglobin molecule, due to molecular aberrations in the α or β genes or their regulatory elements.[28] β Thalassemia is characterized by reduced

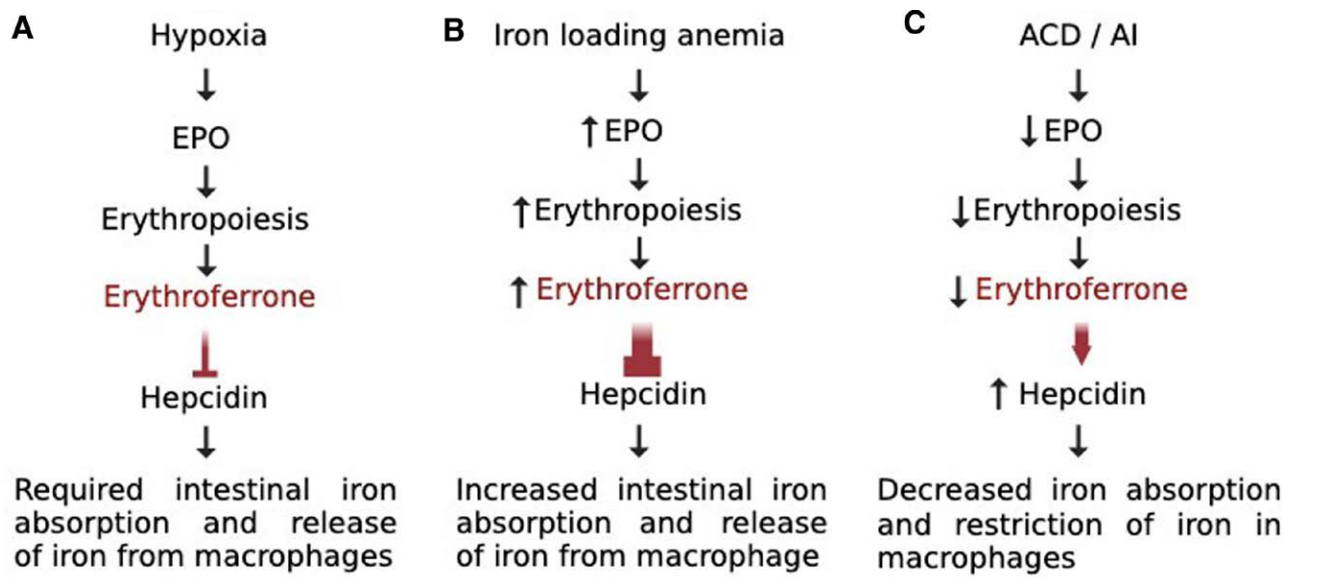

Figure 2. Role of erythroferrone in hypoxia (and anemia), iron loading anemias, and ACD/anemia of inflammation. A: Under normal conditions marked by hypoxia, the kidneys respond by secreting EPO, which stimulates erythroblasts to increase ERFE production. Subsequently, ERFE acts on hepatocytes, suppressing hepcidin production. This, in turn, reduces hepcidin levels, facilitating the release of stored iron from macrophages and intestinal iron absorption thus supporting erythropoiesis. B: In iron-loading anemias such as β-thalassemia, elevated EPO and ERFE production, coupled with chronically low hepcidin levels induced by ERFE, leads to iron overload. In MDS, there is an elevation in ERFE levels in response to proliferative erythropoiesis. However, this elevation becomes counterproductive due to erythroid maturation failure and subsequent iron overload. C: In ACD, inflammatory cytokines, eg, IL-6 has a suppressive effect on EPO production. Elevated IL-6 levels in ACD contribute to the blunted EPO response, leading to inadequate stimulation of erythropoiesis. IL-6 can also induce hepcidin expression causing iron sequestration in macrophages and hepatocytes, exacerbating iron-restricted erythropoiesis and contributing to anemia. ACD = anemia of chronic disease, EPO = erythropoietin, ERFE = erythroferrone, IL = interleukin, MDS = myelodysplastic syndromes.

(β^{+}) or complete absence (β^{0}) of β globin chains synthesis, precipitating a state of ineffective erythropoiesis, shortened red cell survival with subsequent anemia, elevated levels of EPO, and consequent secondary iron overload.[28] The later sequela is frequently exacerbated by the imperative necessity for repeated transfusions.[29]

The paramount etiological factor underpinning morbidity and mortality within individuals afflicted by transfusion-dependent thalassemia major resides in the pernicious phenomenon of iron overload. Unlike most other essential metals, human physiology lacks a dedicated pathway for iron excretion. This vulnerability to iron overload is particularly concerning in the context of ineffective erythropoiesis or repeated blood transfusions, where iron accumulates relentlessly.[30] This surplus iron progressively accumulates within vital body organs such as liver, heart, endocrine glands, thereby precipitating a cascade of functional impairments in these organs.[5,24]

Within the spectrum of β-thalassemia, the uneffected α globin gene continues to synthesize normal quantity of α globin chains leading to a conspicuous excess of α chains that aggregate and precipitate in erythroblasts. The aggregation of α globin chains elicits substantial damage to the cell membrane and induces the generation of reactive oxygen species (ROS), thereby triggering a cascade of events that culminates in ineffective erythropoiesis. This condition is marked by an expansive proliferation of immature erythroblasts, juxtaposed with the apoptosis of mature erythroblasts at polychromatophilic stage, ultimately leading to decreased production of red cells.[31] This ineffective erythropoiesis intricately intertwines with iron overload, a consequence of increased iron absorption and non–transferrin-bound iron (NTBI).[31,32] Such an interplay significantly perturbs erythropoietic regulation.[33] The resultant erythroid hyperplasia leads to excessive synthesis of ERFE which exerts suppression of hepcidin. Consequently, iron stores are mobilized, increasing plasma iron levels and fostering increased absorption of iron within the intestinal milieu (**Fig. 2, B**).[24] The complex interaction between ineffective erythropoiesis and iron dysregulation in β-thalassemia not only contributes significantly to our understanding of the development and progression of anemia in this disease but also highlights the complex nature of iron metabolism within this complex condition. By elucidating this intricate interplay, it is possible to develop targeted therapeutic strategies to address the core pathophysiology of β-thalassemia and improve patient outcomes.[31]

In a murine model of β-thalassemia ($Hbb^{HbbTh3/+}$), ablation of the *Erfe* gene restored hepcidin expression to levels akin to those observed in wild-type mice, concurrently ameliorating tissue iron overload. The augmentative impact of ERFE on hepcidin expression is notably pronounced in juvenile $Hbb^{HbbTh3/+}$ mice, preceding the age-related escalation of liver iron concentration. In contrast, older $Hbb^{HbbTh3/+}$ mice exhibited hepcidin levels comparable to wild-type counterparts; however, these levels remained disproportionately low considering the extensive liver iron accumulation, which under normal circumstances, would elevate hepcidin expression.[9] Furthermore, the capacity of ERFE to modulate hepcidin expression in this model of persistently ineffective erythropoiesis underscores its influence not only in acute erythropoietic scenarios, such as following a single EPO administration or phlebotomy, but also under chronic conditions.[1]

Emerging therapeutic strategies targeting ERFE inhibition hold promise for circumventing iron overload in β-thalassemia and other anemias characterized by ineffective erythropoiesis. By targeting ERFE activity, these novel approaches aim to restore iron homeostasis, potentially offering a unique and valuable tool for managing iron dysregulation in these challenging conditions.[34]

In the complex landscape of β-thalassemia, ERFE has emerged not only as a critical regulator of iron metabolism but also as a key mediator in bone homeostasis. Conjointly, ERFE manifests an osteoprotective effect by intricately modulating BMP signaling within osteoblasts, thereby diminishing the production of Receptor Activator of Nuclear factor Kappa-B Ligand (RANKL). This modulation serves to curtail osteoclastogenesis, effectively averting excessive bone resorption amidst the proliferative surge of erythropoiesis characteristic of β-thalassemia.[35]

3.2. Myelodysplastic syndromes

Myelodysplastic syndromes (MDS) encompass a spectrum of heterogeneous hematopoietic disorders characterized by ineffective erythropoiesis, leading to anemia, neutropenia, and thrombocytopenia.[36] The underlying pathogenesis of MDS is complex and involves a multitude of factors, including genetic abnormalities, epigenetic alterations, and dysregulated signaling pathways.[37] In MDS, erythropoiesis is disrupted, leading to the production of abnormally immature dysplastic erythrocytes. This ineffective erythropoiesis is attributed to several factors, including impaired EPO responsiveness, defective iron utilization, and abnormalities in iron regulatory proteins.[38] In MDS, dysregulated iron metabolism leads to iron accumulation in macrophages and a relative deficiency of iron in erythroid precursors. This iron dysregulation contributes to ineffective erythropoiesis and anemia.[39,40] In patients with MDS, erythropoietin receptor-positive (EPO-R+) bone marrow cells with ring sideroblasts produce high levels of ERFE. High ERFE levels lead to decreased hepcidin expression in hepatocytes, which in turn increases iron absorption and availability for erythropoiesis.[41] The data indicate a significant positive correlation between ERFE and ferritin, along with a negative correlation with hepcidin and the hepcidin/ferritin ratio. This suggests the involvement of ERFE in MDS-related anemia and iron toxicity (**Fig. 2, B**).[42] Elevated levels of ERFE were observed in patients with severe anemia compared to those with moderate anemia, indicating a stronger erythropoietic stimulus. Consequently, no negative correlation between ERFE and hepcidin was found in either patient group, highlighting the disrupted iron metabolism characteristic of MDS.[43]

3.3. Anemia of chronic disease

ACD and AI are 2 prevalent and interconnected forms of anemia characterized by inadequate erythropoiesis. ACD and AI are characterized by a complex interplay between inflammation and iron dysregulation, leading to impaired erythropoiesis. In ACD, chronic inflammation triggered by various conditions, such as infections, autoimmune diseases, and cancer, leads to a cascade of events that suppress erythropoiesis. ILs, a group of pro-inflammatory cytokines, play a central role in this process.[44]

The pathophysiology of these anemias is complex and multifactorial, involving impaired iron utilization, erythropoiesis, and inflammatory cytokines. In ACD, the body's iron regulatory mechanisms are disrupted, leading to sequestration of iron in macrophages and hepatocytes and decreased availability for erythropoiesis. Despite the presence of anemia, there is often a blunted response to EPO, leading to inadequate erythropoiesis. Cytokines such as IL-6, tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ) play significant roles in the pathogenesis of these anemias. TNF-α, IFN-γ, and IL-1 precipitate a disruption in the expression and signaling of the EPO receptor, primarily through the attenuated expression of Scribble (Scb). This modulation leads to the inhibition of EPO and ERFE production. Concurrently, these cytokines exert a direct inhibitory influence on the differentiation and proliferation of erythroid progenitor cells, further impacting erythropoiesis. They not only inhibit erythropoiesis but also increase hepcidin production, further exacerbating iron sequestration (**Fig. 2, C**). IL-6, a primary mediator of inflammation, is elevated in ACD and contributes to hepcidin upregulation.[4,44] Hepcidin withholds iron within macrophages, limiting its availability for

erythropoiesis.[45,46] This iron-restricted erythropoiesis is further exacerbated by reduced EPO production.[44]

AI, on the other hand, arises from direct inflammatory suppression of erythropoiesis. IL-1, another pro-inflammatory cytokine, plays a significant role in AI by directly inhibiting EPO production and erythroid progenitor cell differentiation. Additionally, IL-1 can induce hepcidin expression, further contributing to iron dysregulation.[47]

3.4. Chronic kidney diseases

Chronic kidney disease (CKD) is a significant global health issue affecting millions worldwide.[48] CKD can lead to several complications, including microcytic hypochromic anemia, primarily driven by a deficiency in secondary EPO production due to reduced kidney function. Additionally, CKD disrupts iron homeostasis, characterized by a key contributor: significantly elevated levels of hepcidin. This increase in hepcidin reduces iron release from storage, restricting iron availability for erythropoiesis, and further exacerbating the anemia.[49] This condition leads to functional iron deficiency, where iron is available in the body but not adequately utilized for red blood cell production. Managing anemia in CKD is challenging and often requires a combination of EPO therapy and iron supplementation, tailored to individual patient needs. To manage anemia in CKD, patients often receive EPO therapy or erythropoiesis-stimulating agents (ESAs). When erythropoiesis is stimulated, either naturally or through EPO therapy, erythroblasts in the bone marrow increase the production of ERFE.[50]

In patients with CKD, the role of IL-6 in iron metabolism is crucial and multifaceted. IL-6 is often elevated in CKD, contributing to the inflammatory state commonly observed in these patients. Increased IL-6 levels have direct implications for iron metabolism. Primarily, IL-6 stimulates the production of hepcidin. Elevated hepcidin levels lead to decreased iron absorption from the gut and the retention of iron within macrophages and hepatocytes.[4] This process effectively reduces the availability of iron for erythropoiesis, contributing to the anemia in CKD patients. Furthermore, the IL-6–induced increase in hepcidin can exacerbate the functional iron deficiency in patients with CKD, where there is iron in the body, but it is not adequately mobilized for effective erythropoiesis.[51] This mechanism highlights the complex interplay between inflammation, iron metabolism, and anemia in CKD, underscoring the importance of managing inflammatory processes to improve iron availability and anemia outcomes in these patients.

3.5. Iron deficiency anemia

Iron deficiency anemia (IDA) is one of the most prevalent nutritional deficiencies worldwide.[52] In the milieu of iron deficiency, ERFE emerges as a pivotal hematological arbiter, exhibiting a marked upsurge in systemic concentration, a phenomenon intricately linked to the compensatory erythropoietic activity. This elevation in ERFE articulates an inverse relationship with key iron indices such as serum ferritin and transferrin saturation, while concurrently demonstrating a direct correlation with total iron binding capacity (TIBC), thereby underscoring its integral role in the intricate regulatory nexus of iron homeostasis under conditions of depleted iron reserves.[53,54]

3.6. Hereditary hemochromatosis

It is a genetic disorder characterized by excessive iron absorption and accumulation. The genes *HFE*, *HJV* (encoding hemojuvelin), *HAMP* (encoding hepcidin), and *TFR2* (encoding transferrin receptor 2) constitute critical components of the hepcidin-activating pathway in hepatocytes. Autosomal-recessive inactivation of any one of these genes results in a

Table 1

Role of ERFE, its effects on iron metabolism, and the therapeutic implications in dysregulated iron metabolic disorders.

Disease	Key characteristics	Role of ERFE	Impact on iron metabolism	Therapeutic implications
β-T	Genetic disorder with reduced/absent β-globin chains	Elevated ERFE levels to suppress hepcidin	Causes iron overload due to ineffective erythropoiesis	Potential target for reducing iron overload
MDS	Clonal hematopoietic stem cell disorders	Elevated ERFE levels without effective hepcidin suppression	Disrupted iron metabolism with variable iron overload	ERFE modulation may assist in managing iron overload
ACD	Anemia associated with chronic diseases	Disrupted ERFE regulation by inflammatory cytokines	Increased hepcidin levels lead to iron sequestration in macrophages	Targeting ERFE may help manage iron sequestration
AI	Anemia due to chronic inflammatory conditions	Elevated ERFE to compensate for inflammation-induced anemia	Increased hepcidin levels lead to impaired iron utilization	Targeting ERFE may help in managing anemia
CKD	Progressive loss of kidney function	Reduced ERFE due to impaired EPO production	Disrupted iron homeostasis, often with anemia due to reduced erythropoiesis	ERFE administration could potentially improve anemia management
IDA	Nutritional deficiency resulting in low iron levels	Elevated ERFE levels to enhance iron availability	Inverse relationship with serum ferritin and transferrin saturation; direct correlation with TIBC	Diagnostic marker and potential therapeutic target
HH	Genetic disorder causing excessive iron absorption	Insufficient ERFE response leading to hepcidin deficiency	Severe iron overload due to low hepcidin levels	ERFE-based therapies might help restore iron balance

β-T = β-Thalassemia, ACD = anemia of chronic disease, AI = anemia of inflammation, CKD = chronic kidney disease, ERFE = erythroferrone, HH = hereditary hemochromatosis, IDA = iron deficiency anemia, MDS = myelodysplastic syndrome.

notable deficiency of hepcidin, culminating in systemic iron overload or hemochromatosis. Functional investigations of these genes have elucidated that BMP signaling, mediated through the phosphorylation of SMAD proteins, stands as a central regulatory pathway for the transcription of hepcidin.[55,56]

The relationship between hereditary hemochromatosis and ERFE is an area of emerging interest and research. In the context of hereditary hemochromatosis, the regulatory balance between ERFE and hepcidin might be disrupted. The mutations associated with hemochromatosis can lead to inappropriately low levels of hepcidin, resulting in increased intestinal iron absorption and accumulation in the body. The role of ERFE in this scenario may be complex. On one hand, increased erythropoietic activity due to iron overload might stimulate ERFE production, which could further suppress hepcidin levels and exacerbate iron accumulation. On the other hand, the overall contribution of ERFE in the setting of the genetic mutations that characterize hereditary hemochromatosis is still not fully understood. Understanding the interplay between ERFE and hepcidin in hereditary hemochromatosis could provide new insights into the pathophysiology of this disorder and potentially lead to novel therapeutic strategies. For example, modulating ERFE activity could be a potential approach to correct the imbalance in hepcidin levels and iron metabolism in affected individuals. Table 1 depicts the role of ERFE, its impact on iron metabolism, and the therapeutic implications in the diseases discussed earlier.

4. DIAGNOSTIC AND THERAPEUTIC INSIGHTS

The determination of ERFE levels can provide diagnostic clarity in iron disorders. In diseases, like β-thalassemia, therapeutic strategies aimed at normalizing ERFE levels could help manage iron overload. Conversely, in ACD or IDA, strategies to modulate ERFE could improve treatment efficacy. Modulating ERFE levels in iron overload disorders offers a novel therapeutic approach. By reducing ERFE levels, it may be possible to restore hepcidin function, thereby managing iron levels effectively.

5. CONCLUSION

In conclusion, ERFE plays a critical role in iron metabolism, particularly in conditions like β-thalassemia and ACD. Its regulatory influence on hepcidin underscores its potential as a therapeutic target for managing iron overload disorders. Continued investigation of ERFE's functions promises to enhance our understanding and treatment of hematological diseases characterized by dysregulated iron homeostasis.

AUTHOR CONTRIBUTIONS

S.B. carried out the literature search and drafted the manuscript. M.S. edited, critically revised the work, and finalized the manuscript.

REFERENCES

[1] Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T. Identification of erythroferrone as an erythroid regulator of iron metabolism. *Nat Genet* 2014;46(7):678–684.

[2] Ganz T. Erythropoietic regulators of iron metabolism. *Free Radic Biol Med* 2019;133:69–74.

[3] Kautz L, Jung G, Nemeth E, Ganz T. The erythroid factor erythroferrone and its role in iron homeostasis. *Blood* 2013;122(21):4–4.

[4] Nemeth E, Rivera S, Gabayan V, et al. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest* 2004;113(9):1271–1276.

[5] El-Gamal RAE-R, Abdel-Messih IY, Habashy DM, Zaiema SEG, Pessar SA. Erythroferrone, the new iron regulator: evaluation of its levels in Egyptian patients with beta thalassemia. *Ann Hematol* 2020;99(1):31–39.

[6] Roth M-P, Meynard D, Coppin H. Regulators of hepcidin expression. *Vitam Horm* 2019;110:101–129.

[7] Seldin MM, Peterson JM, Byerly MS, Wei Z, Wong GW. Myonectin (CTRP15), a novel myokine that links skeletal muscle to systemic lipid homeostasis. *J Biol Chem* 2012;287(15):11968–11980.

[8] Coffey R, Ganz T. Erythroferrone: an erythroid regulator of hepcidin and iron metabolism. *Hemasphere* 2018;2(2):e35.

[9] Kautz L, Jung G, Du X, et al. Erythroferrone contributes to hepcidin suppression and iron overload in a mouse model of β-thalassemia. *Blood* 2015;126(17):2031–2037.

[10] Balakrishnan R, Thurmond DC. Mechanisms by which skeletal muscle myokines ameliorate insulin resistance. *Int J Mol Sci* 2022;23(9):4636.

[11] Little HC, Rodriguez S, Lei X, et al. Myonectin deletion promotes adipose fat storage and reduces liver steatosis. *FASEB J* 2019;33(7):8666–8687.

[12] Berezin AE, Berezin AA, Lichtenauer M. Myokines and heart failure: challenging role in adverse cardiac remodeling, myopathy, and clinical outcomes. *Dis Markers* 2021;2021(6644631):1–17.

[13] Srole DN, Ganz T. Erythroferrone structure, function, and physiology: iron homeostasis and beyond. *J Cell Physiol* 2021;236(7):4888–4901. doi:10.1002/jcp.30247.

[14] Blom N, Sicheritz-Pontén T, Gupta R, Gammeltoft S, Brunak S. Prediction of post-translational glycosylation and phosphorylation of proteins from the amino acid sequence. *Proteomics* 2004;4(6):1633–1649.

[15] Srole DN, Jung G, Waring AJ, Nemeth E, Ganz T. Characterization of erythroferrone structural domains relevant to its iron-regulatory function. *J Biol Chem* 2023;299(12):105374. doi:10.1016/j.jbc.2023.105374.

[16] Wang Y, Lam KSL, Yau M, Xu A. Post-translational modifications of adiponectin: mechanisms and functional implications. *Biochem J* 2008;409(3):623–633.

[17] Haase VH. Regulation of erythropoiesis by hypoxia-inducible factors. *Blood Rev* 2013;27(1):41–53.

[18] Hodges VM, Rainey S, Lappin TR, Maxwell AP. Pathophysiology of anemia and erythrocytosis. *Crit Rev Oncol Hematol* 2007;64(2):139–158.

[19] Saboor M. Hepcidin: structure, functions and role in hematological disorders. *Majmaah J Heal Sci* 2021;9(3):121–136.

[20] Wojciechowska M, Wisniewski OW, Kolodziejski P, Krauss H. Role of hepcidin in physiology and pathophysiology. Emerging experimental and clinical evidence. *J Physiol Pharmacol* 2021;72(1):23–33.

[21] Xu Y, Alfaro-Magallanes VM, Babitt JL. Physiological and pathophysiological mechanisms of hepcidin regulation: clinical implications for iron disorders. *Br J Haematol* 2021;193(5):882–893.

[22] Girelli D, Nemeth E, Swinkels DW. Hepcidin in the diagnosis of iron disorders. *Blood* 2016;127(23):2809–2813.

[23] Sangkhae V, Nemeth E. Regulation of the iron homeostatic hormone hepcidin. *Adv Nutr* 2017;8(1):126–136.

[24] Moura IC, Hermine O. Erythroferrone: the missing link in β-thalassemia? *Blood* 2015;126(17):1974–1975.

[25] Arezes J, Foy N, McHugh K, et al. Erythroferrone inhibits the induction of hepcidin by BMP6. *Blood* 2018;132(14):1473–1477.

[26] Wang CY, Xu Y, Traeger L, et al. Erythroferrone lowers hepcidin by sequestering BMP2/6 heterodimer from binding to the BMP type I receptor ALK3. *Blood* 2020;135(6):453–456.

[27] Xiao X, Xu Y, Moschetta GA, et al. BMP5 contributes to hepcidin regulation and systemic iron homeostasis in mice. *Blood* 2023;142(15):1312–1322.

[28] Baird DC, Batten SH, Sparks SK. Alpha- and beta-thalassemia: rapid evidence review. *Am Fam Physician* 2022;105(3):272–280.

[29] Cazzola M. Ineffective erythropoiesis and its treatment. *Blood* 2022;139(16):2460–2470.

[30] Camaschella C, Nai A. Ineffective erythropoiesis and regulation of iron status in iron loading anaemias. *Br J Haematol* 2016;172(4):512–523.

[31] Rivella S. Iron metabolism under conditions of ineffective erythropoiesis in β-thalassemia. *Blood* 2019;133(1):51–58.

[32] Evans RW, Rafique R, Zarea A, et al. Nature of non-transferrin-bound iron: studies on iron citrate complexes and thalassemic sera. *J Biol Inorg Chem* 2008;13(1):57–74.

[33] Hershko C. Pathogenesis and management of iron toxicity in thalassemia. *Ann N Y Acad Sci* 2010;1202:1–9.

[34] Au TY, Benjamin S, Wiśniewski OW. Is the role of hepcidin and erythroferrone in the pathogenesis of beta thalassemia the key to developing novel treatment strategies? *Thalassemia Rep* 2022;12(3):123–134.

[35] Castro-Mollo M, Gera S, Ruiz-Martinez M, et al. The hepcidin regulator erythroferrone is a new member of the erythropoiesis-iron-bone circuitry. *Elife* 2021;10:1–17.

[36] Saboor M, Moinuddin M. *Hematology for the Undergraduates*. 1st ed. In: Saboor MM, ed. Pakistan: Higher Education Commission; 2015.
[37] Garcia-Manero G. Myelodysplastic syndromes: 2023 update on diagnosis, risk-stratification, and management. *Am J Hematol* 2023;98(8):1307–1325.
[38] Fontenay M, Boussaid I, Chapuis N. Pathophysiology of myelodysplastic syndromes. *Bull Cancer* 2023;110(11):1097–1105.
[39] Shenoy N, Vallumsetla N, Rachmilewitz E, Verma A, Ginzburg Y. Impact of iron overload and potential benefit from iron chelation in low-risk myelodysplastic syndrome. *Blood* 2014;124(6):873–881.
[40] Vinchi F, Hell S, Platzbecker U. Controversies on the consequences of iron overload and chelation in MDS. *Hemasphere* 2020;4(3):e357.
[41] Miura S, Kobune M, Horiguchi H, et al. EPO-R+ myelodysplastic cells with ring sideroblasts produce high erythroferrone levels to reduce hepcidin expression in hepatic cells. *Blood Cells Mol Dis* 2019;78:1–8.
[42] Bondu S, Alary A-S, Lefèvre C, et al. A variant erythroferrone disrupts iron homeostasis in SF3B1-mutated myelodysplastic syndrome. *Sci Transl Med* 2019;11(500):eaav5467.
[43] Oarbeascoa G, Redondo S, Morán-Jiménez MJ, et al. Hepcidin and erythroferrone in the anemia of low-risk myelodysplastic syndromes. *Blood* 2018;132(Supplement 1):3085–3085.
[44] Weiss G, Ganz T, Goodnough LT. Anemia of inflammation. *Blood* 2019;133(1):40–50.
[45] Ganz T, Nemeth E. Iron homeostasis in host defence and inflammation. *Nat Rev Immunol* 2015;15(8):500–510.
[46] Ganz T. Anemia of inflammation. *N Engl J Med* 2019;381(12):1148–1157.
[47] Madu AJ, Ughasoro MD. Anaemia of chronic disease: an in-depth review. *Med Princ Pract* 2017;26(1):1–9.
[48] Gaitonde DY, Cook DL, Rivera IM. Chronic kidney disease: detection and evaluation. *Am Fam Physician* 2017;96(12):776–783.
[49] Spoto B, Kakkar R, Lo L, et al. Serum erythroferrone levels associate with mortality and cardiovascular events in hemodialysis and in CKD patients: a two cohorts study. *J Clin Med* 2019;8(4):523.
[50] Honda H, Kobayashi Y, Onuma S, et al. Associations among erythroferrone and biomarkers of erythropoiesis and iron metabolism, and treatment with long-term erythropoiesis-stimulating agents in patients on hemodialysis. *PLoS One* 2016;11(3):e0151601.
[51] Begum S, Latunde-Dada GO. Anemia of inflammation with an emphasis on chronic kidney disease. *Nutrients* 2019;11(10):2424.
[52] Saboor M, Zehra A, Qamar K, Moinuddin. Disorders associated with malabsorption of iron: a critical review. *Pak J Med Sci* 2015;31(6):1549–1553.
[53] El Gendy FM, El-Hawy MA, Shehata AMF, Osheba HE. Erythroferrone and iron status parameters levels in pediatric patients with iron deficiency anemia. *Eur J Haematol* 2018;100(4):356–360.
[54] Babker AM. Associations between erythroferrone and hepcidin during iron deficiency anemia, iron overload and pregnancy. *Int J Health Sci (Qassim)* 2022;6(4):6171–6177.
[55] Camaschella C. Iron and hepcidin: a story of recycling and balance. *Hematology Am Soc Hematol Educ Program* 2013;2013:1–8.
[56] Camaschella C, Poggiali E. Inherited disorders of iron metabolism. *Curr Opin Pediatr* 2011;23(1):14–20.