

## Review Article



# Roles of microRNAs in thalassemia: Mechanisms, biomarkers, and therapeutic implications – a narrative review

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### Abstract

Thalassemia, a kind of hemoglobin disorder, affects more than 1.5% of people worldwide. Small RNA molecules, known as microRNAs (miRNAs), are factors in controlling gene production, including hemoglobin production and disease development. This comprehensive review delves into the functions of miRNAs in the pathophysiology of thalassemia. The miRNAs miR-16 and miR-222 have been found to influence the dysregulation of erythropoiesis. These miRNAs act in impeding normal erythropoiesis, resulting in inefficient red blood cell (RBC) production and the premature demise of erythroid progenitor cells. Moreover, other miRNAs like miR-150, miR-210, and miR-485-3p regulate iron levels by pointing molecules such as transferrin receptor 1 (TfR1), ferritin, and hepcidin, which can worsen iron overload issues. Additionally, certain miRNAs have been recognized as markers for the identification of complications related to transfusions in individuals with thalassemia. The miRNAs like miR-451a, miR-20a, and miR-21 are associated with conditions such as iron overload and liver damage. Strategies involving the manipulation of the RNA molecules through mimics or inhibitors show promise in rebalancing gene activity and alleviating symptoms associated with thalassemia based on clinical trials conducted previously. The current review sheds light on how these miRNAs play a key role in modulating immune responses, and stress levels, along with organ-specific issues like heart or liver problems in patients with thalassemia. The miRNAs, including those mentioned above, impact immune cell performance, inflammation processes, and fibrosis development, contributing to the emergence of complications following transfusions. In conclusion, this comprehensive narrative review underlines the critical role of miRNAs in the pathogenesis of thalassemia. The findings illuminate their multifaceted potential as diagnostic biomarkers, therapeutic capacities, and cornerstones for developing tailored medical approaches. By harnessing these insights, clinicians and researchers may revolutionize thalassemia management, significantly improving patients' health outcomes and quality of life.

### Introduction

Thalassemia, characterized by reduced or absent hemoglobin production, poses a significant public health issue, affecting 1.5% of the global population who carry a mutated gene in their blood.<sup>1</sup> The condition typically requires regular blood transfusions, leading to potential complications such as excessive iron accumulation and organ damage. The mutations on the beta and alpha genes are a key point that affects the production of the globin chain, an essential element of hemoglobin.<sup>2,3</sup> The alterations interfere normal production of hemoglobin, leading to severe anemia. Small non-coding RNA molecules, microRNAs (miRNAs), provide a crucial mechanism in post-transcriptional control of fine-tuning gene expression and might play a part in managing the genes expression involved in globin synthesis.<sup>4</sup>

Emerging research suggests that miRNAs may be possible biomarkers for predicting and tracking complications in patients with thalassemia, resulting from frequent blood transfusions. Regulatory miRNAs have become an exciting research focus in hemoglobin disorders, with evidence hinting at their possible contributions to the pathogenesis and potential as both diagnostic markers and therapeutic targets. This paper reviewed current knowledge on the functions of miRNAs in these conditions. It was structured into sections examining miRNAs' diverse roles, like their potential as markers of transfusion complications, possible therapeutic uses, and effects on immunity, oxidative stress, iron, cardiac and liver function, red blood cell (RBC) formation, and bone marrow. Each section summarized recent studies on how miRNAs could impact different facets of the development,

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worsening, and management of hemoglobin disorders.<sup>5</sup> A great body of studies has also identified specific miRNAs, regulating gene globin synthesis in the thalassemia process. Loss of control of miR-144 can lead to the silencing of embryonic  $\alpha$ -globin and  $\zeta$ -globin gene expression in human erythroid cells by targeting the erythroid-specific transcription factor KLF1.<sup>6,7</sup> A study designed by Lulli and colleagues revealed that microRNA-486-3p (miR-486-3p) functions as a modulator of  $\gamma$ -globin gene expression in human RBC precursors. Their research indicated that this regulation occurs through the alteration of BCL11A levels, a known repressor of  $\gamma$ -globin, suggesting its potential role in the clinical severity of thalassemia.<sup>8</sup> Furthermore, dysregulation of miRNAs and related genes has been observed in pediatric beta-thalassemia, highlighting the utility as diagnostic and prognostic biomarkers various conditions.<sup>5</sup> A major component of thalassemia managing involves dealing with complications from frequent blood transfusions, including iron overload and immune reactions.<sup>9,10</sup> Recent research indicates that miRNAs could serve as biomarkers to anticipate and track transfusion-associated complications. Altering miRNAs represents a thrilling and promising tactic for advancing new thalassemia therapeutic schedules by modulating various pathways. Potential modalities include using synthetic miRNA analogs, miRNA inhibitors, and gene editing approaches.<sup>11,12</sup> This article outlined current research on miRNAs' involvement in thalassemia, exploring their impact on disease mechanisms and potential applications in diagnosis and treatment. The key questions we seek to address include (1) How do specific miRNAs contribute to the dysregulation of erythropoiesis and ineffective erythropoiesis observed in thalassemia? (2) What is the potential of miRNAs for predicting and monitoring transfusion-related complications in thalassemia patients? (3) How can miRNA modulation strategies be leveraged for developing novel therapeutic approaches for thalassemia? (4) What are the mechanisms by which miRNAs influence immune responses, oxidative stress, iron metabolism, and organ-specific complications, such as cardiac and liver dysfunction, in thalassemia patients? By systematically examining the available evidence, we aimed to elucidate the multifaceted roles of miRNAs in thalassemia and explore their potential as diagnostic markers, therapeutic targets, and avenues for personalized medicine to improve patient outcomes.

## Methods

### Literature search strategy

We performed a comprehensive search on the present literature to identify relevant studies. Data was collected by conducting searches in a common database, including PubMed, Science Direct, and Google Scholar, with English language and no limitation on date publication. The search terms used were a combination of keywords related to "microRNA," "thalassemia," "erythropoiesis,"

"iron metabolism," "oxidative stress," "immune response," "cardiac function," "liver function," "biomarkers," and "therapeutic targets." Additionally, the reference lists of related review articles and included studies were manually screened to identify any additional pertinent publications.

### Inclusion and exclusion criteria

Studies were included if they met requirements as follows: (1) original research articles or review articles focusing on the roles of miRNAs in thalassemia pathophysiology, diagnosis, or treatment; (2) All *in vitro*, *in vivo*, and clinical studies performed on miRNAs effects in thalassemia; (3) studies reporting the association between specific miRNAs and thalassemia aspects, such as erythropoiesis, iron metabolism, oxidative stress, immune response, cardiac or liver function, transfusion-related complications, or potential therapeutic implications. Studies were excluded if they were non-English publications, case reports, commentaries, letters to the editor, or articles not relevant to the topic of miRNAs and thalassemia.

### Study selection and data extraction

Two reviewers (LR and TW) independently screened the titles and abstracts of the retrieved records to identify potentially relevant studies. Then, the full-text of articles were obtained for the selected studies and evaluated against the inclusion and exclusion criteria. Data from the included studies were extracted using a common extraction form, which captured information on the study design, participant characteristics, miRNAs investigated, key findings, and conclusions.

### Data synthesis and presentation

The extracted data were synthesized qualitatively and organized into subsections based on the specific roles and mechanisms of miRNAs in thalassemia, including their effects on erythropoiesis, iron metabolism, oxidative stress, immune response, cardiac and liver function, and their potential as diagnostic indicators, as well as targets for therapeutic development. Relevant findings from the included studies were summarized in each subsection, and Table 1 was designed to provide an overview of the key miRNAs and their associated functions or implications in thalassemia.

## Results

The literature search yielded a total of 375 records from the electronic databases and additional records identified through manual reference screening. After removing duplicates and screening titles and abstracts, 107 full-text articles were assessed for eligibility. Of these, 97 research papers satisfied the predefined selection parameters and were incorporated into the qualitative analysis. The comprehensive analysis noted that a wide array of miRNAs play crucial roles in various aspects of thalassemia pathophysiology as depicted in Table 1.

**Table 1.** List of the miRNAs along with their biological aspects including in thalassemia pathophysiology

miRNAs	Biological aspects
miR-16	Regulating normal erythropoiesis; downregulated in beta-thalassemia leading to erythroid hyperplasia.
miR-451	Regulating normal erythropoiesis; downregulated in beta-thalassemia leading to erythroid hyperplasia.
miR-221	Upregulated in alpha-thalassemia, leading to erythroid cell apoptosis
miR-222	Upregulated in alpha-thalassemia, leading to erythroid cell apoptosis
miR-210	Suppressing erythropoiesis in alpha-thalassemia; modulating oxidative stress.
miR-150	Inhibiting proliferation and differentiation of erythroid progenitor cells; modulating immune cell function (T and B lymphocytes); potential biomarker for cardiac dysfunction
miR-485-3p	Regulating iron metabolism by targeting transferrin receptor 1 (TfR1)
miR-20a	Regulating iron metabolism by targeting ferritin; a potential biomarker for iron overload and liver damage
miR-451a	Potential biomarker for iron overload, liver fibrosis, and hemolysis/transfusion reactions
miR-21	Potential biomarker for iron overload and liver damage
miR-122	Regulating hepcidin and iron metabolism; involved in liver function and diseases.
miR-27b	Inhibiting ubiquitin-specific peptidase 4 (USP4), Shielding liver cells from the effects of the TGF- $\beta$ signaling route; potential therapeutic target for liver fibrosis.
miR-23b	Part of the miR-23b/27b/24-1 cluster, suppresses the fibrotic of the liver.
miR-27b	Part of the miR-23b/27b/24-1 cluster, suppresses the fibrotic of the liver.
miR-24-1	Part of the miR-23b/27b/24-1 cluster, suppresses the fibrotic of the liver.
miR-765	Regulating cardiomyocyte contractile function and calcium cycling
miR-155	Modulating left ventricular mass index; potential therapeutic target for heart failure; modulating immune reactions associated with transfusions
miR-212	Potential therapeutic target for pathological cardiac remodeling and heart failure
miR-132	Potential therapeutic target for pathological cardiac remodeling and heart failure
miR-652	Potential therapeutic target for pathological cardiac remodeling and heart failure
miR-34a	Potential therapeutic target for dilated cardiomyopathy (sex-specific effects)
miR-25	Promoting cardiomyocyte proliferation by targeting FBXW7; a potential target for cardiac regeneration.
miR-30d	Regulating cardiac function through intracellular and paracrine signaling
miR-126	Regulating angiogenesis and vascular integrity; potential therapeutic target for myocardial infarction
miR-146a	Regulating angiogenesis and vascular integrity; potential therapeutic target for myocardial infarction
miR-let-7b	Regulating ferroportin and iron metabolism
miR-155	Modulating immune reactions associated with transfusions
miR-146a	Potential biomarker for inflammation-related complications like sepsis
miR-223	Potential biomarker for inflammation-related complications like sepsis
miR-92a-3p	Associated with hemolysis/transfusion reactions
miR-16-5p	Associated with hemolysis/transfusion reactions
miR-let-7b	Controlling inflammation by regulating the NF- $\kappa$ B pathway
miR-155	Controlling inflammation by regulating the NF- $\kappa$ B pathway
miR-146a	Controlling inflammation by regulating the NF- $\kappa$ B pathway
miR-144	Increased sensitivity to oxidative stress
miR-214	Upregulated in thalassemia patients with oxidative stress
miR-92a-3p	Regulating gamma-globin, GSH, SOD, ROS, and cell apoptosis
miR-9	Suppressing FoxO3 and affecting ROS production

## Discussion

The following subsections will delve into the certain pathways by which miRNAs contribute to the dysregulation of erythropoiesis, modulate iron metabolism, serve as potential biomarkers for transfusion-related complications, and offer therapeutic prospects through miRNA modulation strategies. Additionally, the intricate interplay between

miRNAs and immune responses, oxidative stress, and organ-specific complications in thalassemia patients will be explored. Here, we aim to underscore the significance of miRNAs as key factors in thalassemia pathogenesis, potential diagnostic biomarkers, and promising therapeutic targets, ultimately paving the way for novel personalized medicine approaches and improved patient outcomes.

### ***Biogenesis of miRNAs***

MicroRNAs are short non-coding RNA nucleotides (18-24 nucleotides long) found in untranslated regions of mRNA, regulate gene expression at a particular base sequence in mRNA, which results in either mRNA degradation or translation inhibition, thereby influencing essential cellular activities such as cell propagation, apoptosis, and differentiation.<sup>13-15</sup> The gene will lose activity due to binding to the target mRNA. Several studies have shown that miRNAs have multiple targets involved in several diseases associated with multiple cellular processes and play a role in tumorigenesis, cardiovascular disease, diabetes, thalassemia, and others.<sup>5,16-18</sup> In humans, miRNAs are found in all chromosomes, except the Y chromosome. RNA Polymerase II facilitates the primary transcription of these mRNA sequences into hairpin structures called Pri-miRNAs, which are transcribed from the intronic region.<sup>19</sup> Within the nucleus, the Drosha type of RNase III and other associated proteins form the Drosha complex, responsible for cleaving the pri-miRNAs into pre-miRNA. Subsequently, Exportin-5 and RanGTP facilitate the transport of pre-miRNA, a nascent miRNA transcript translocated from the nuclear compartment to the cytosolic milieu. In the cytoplasm, Dicer, an RNase III endonuclease, will process the duplex pre-miRNA into a single mature miRNA strand. The single-strand miRNA then binds to the target mRNA influencing its translation pathway and determining whether the mRNA will be translated or degraded.

### ***Role of miRNAs in thalassemia***

Thalassemia is an inherited disease characterized by impaired synthesis of the beta or alpha globin chains of hemoglobin leading to chronic hemolytic anemia,<sup>20</sup> and an increase in the number of early erythroid precursors and erythroid progenitors, which are immature cells involved in the production of RBCs. Despite the abundance of immature cells, erythropoiesis remains ineffective, resulting in suboptimal production of mature RBCs. Although there is a large pool of proliferating immature erythroblasts, the process of their maturation into functional red cells is impaired.<sup>21</sup>

Ineffective RBC production (erythropoiesis) results from two key factors: the premature death of erythroid precursors via apoptosis and, as recent studies reveal, impaired cell differentiation. This dual problem not only worsens anemia but also triggers increased iron absorption due to the body's compensatory process. However, the dysfunctional erythropoiesis cannot utilize this extra iron effectively, promoting an iron overload and possible organ failure.<sup>22</sup> Research has revealed that miRNAs are pivotal regulators in the aberrant mechanisms underlying thalassemia, particularly in the disruption of normal RBC formation processes. miR-16 and miR-451 are two molecules regulating normal erythropoiesis. In beta-thalassemia, downregulation of

miR-16 and miR-451 leads to erythroid hyperplasia,<sup>3,23</sup> which is typically observed in the bone marrow biopsy of individuals with ineffective erythropoiesis, and serves as a mechanism that help maintain a relatively stable RBC count, albeit abnormal size, shape, or function.

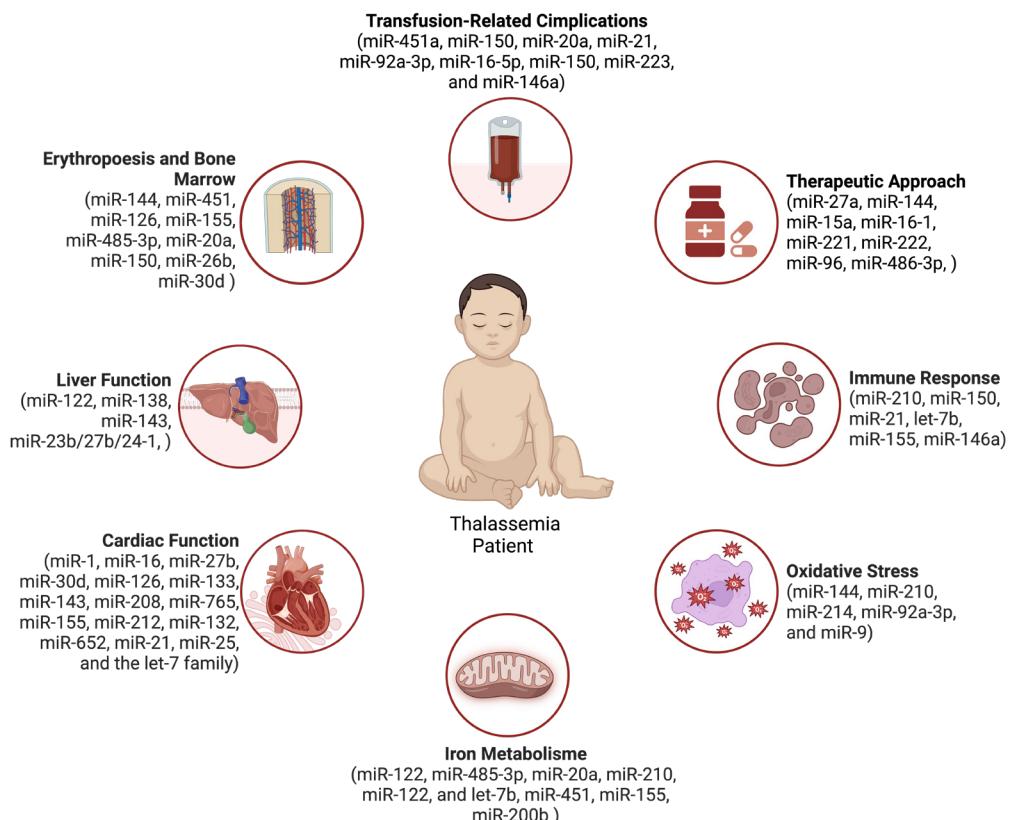
In α-thalassemia, miR-16 and miR-451 have been shown to target the 3' untranslated region (3'-UTR) of the α-globin mRNA by decreasing its stability and translation. The study also found that miR-221 was upregulated, leading to erythroid cell apoptosis as well as miR-222,<sup>24</sup> while miR-210 has been implicated in suppressing erythropoiesis, which disrupted the RBCs' formation process in α-thalassemia.<sup>25-27</sup> Several miRNAs have been also identified as negative regulators of erythropoiesis. For example, miR-150 has the potential to inhibit the proliferation and differentiation of erythroid progenitor cells by targeting c-Myb, a transcription factor critical for erythropoiesis.<sup>26,28</sup> Similarly, miR-221/222 has been shown to inhibit erythroid differentiation by targeting the stem cell factor receptor c-kit.<sup>29</sup>

The miR-210 has been also shown to suppress erythropoietin (EPO), a hormone stimulates erythropoiesis under hypoxia condition.<sup>30</sup> This miRNA is up-regulated in response to hypoxia and targets the 3'-UTR of the EPO mRNA, leading to its degradation and decreased production of EPO. Another finding confirmed that upregulation of miR-210 can affect the globin gene expression and erythroid differentiation in β-thalassemia/HbE erythroid progenitor.<sup>31</sup>

Additionally, long non-coding RNAs and circular RNAs, emerging evidence points to the involvement of alternative non-coding RNA species, play a role in modulating and regulating erythropoiesis. The lncRNA erythroid differentiation regulator (ERDR1) also promote erythroid differentiation by interacting with the transcription factor GATA1, which is critical for erythropoiesis.<sup>32</sup> miRNA as systemic biomarkers in thalassemia will be discussed further in the following subsection, while Figure 1 summarizes the miRNAs involved in various metabolic pathways or systemic effects in thalassemia patients.

### ***miRNAs: as biomarkers for transfusion-related complications***

Transfusion therapy is an essential treatment for many patients with thalassemia, but it carries the risk of various complications, including iron overload, alloimmunization, and infections.<sup>33-35</sup> There is a pressing need to establish biomarkers with potential to predict these complications at the early stage. The development of such diagnostic indicators would allow for more pre-emptive screening for transfusion-associated complications, facilitating timely interventions and improved patient outcomes. More research is warranted to identify novel biomarkers that are accessible, rapid, and reliable for the early diagnosis of complications following blood transfusions.<sup>16</sup>



**Figure 1.** miRNA and metabolism influence in Thalassemia patients

Multiple studies have also shown altered miRNA expression in thalassemia patients with complications, indicating their potential use as diagnostic biomarkers. Specific miRNAs like miR-451a, miR-20a, and miR-21 have been found upregulated in patients with iron overload and liver fibrosis.<sup>36</sup> miR-150 may serve as a biomarker for cardiac dysfunction.<sup>37</sup> The miRNAs have been found to influence gene expression and contribute to various pathological conditions, notably the complications arising from frequent blood transfusions.<sup>38-40</sup> Studies have revealed associations between miRNAs like miR-451a, miR-92a-3p, miR-16-5p, and hemolysis/transfusion reactions.<sup>41-43</sup>

Additionally, miR-150, miR-223, and miR-146a may have utility as markers for inflammation-related complications like sepsis. Overall, miRNAs can be considered to accessible and non-invasive biomarkers for the early detection of transfusion-related complications in thalassemia. More research is also needed to translate these findings into clinical settings.<sup>44-47</sup>

miRNAs have multiple benefits compared to conventional biomarkers for detecting transfusion complications, such as stability in plasma and serum, as well as enabling precise and sensitive quantification. They can also obtain through minimally invasive techniques.<sup>48</sup> Therefore, miRNAs could enhance diagnosis, treatment, and prognosis for transfusion-related complications.

However, more studies are necessary to confirm the clinical importance of miRNA for this purpose. Standardizing methods for miRNA isolation, measurement, and normalization is also essential to ensure result consistency and reproducibility. Despite many challenges, miRNAs have the remarkable potential to transform diagnosis and management of transfusion complications, ultimately improving patient outcomes.<sup>49</sup> Following analytical and clinical validation, miRNAs may be regarded as standard biomarkers for early and non-invasive detection of complications.

#### **miRNAs and immune response**

Numerous studies have detected changes in miRNA expression patterns in patients with thalassemia who develop immune-mediated issues like infections, autoimmunity, and transfusion reactions. The dysregulated miRNA profiles in these patients highlight the substantial role of miRNAs in modulating immune cell function and inflammatory processes that drive the immune-related comorbidities of thalassemia. As well, the miRNAs have broad effects on multiple aspects of immune cell biology, including development, differentiation, activation, cytokine production, cell migration, and antigen presentation. By regulating these key immunological processes, miRNAs are intricately involved in orchestrating both innate and adaptive

immune responses.<sup>50-53</sup> Upregulation of miRNAs like miR-144, miR-451, and miR-503 disrupts normal erythropoiesis, leading to ineffective erythropoiesis, that triggers inflammatory responses and oxidative stress, which can impair immune cell function and promote tissue damage.<sup>4,54</sup> Specific miRNAs have been shown to alter immune cell activity in pathological hematology patients, contributing to inflammatory and oxidative stress responses. In previous research reported that miR-210 and miR-150 can modulate the function of T and B lymphocytes, which are critical mediators of immune reactions.<sup>55</sup> Elevated levels of miR-210 suppress the cytokine production triggered by LPS exposure like IL-6, TNF- $\alpha$ , IL-1 $\beta$ , and CCL2 by targeting NF- $\kappa$ B signaling.<sup>56</sup> microRNA-150 plays a regulatory role in the maturation of natural killer cells and invariant natural killer T cells, two distinct immune cell populations with cytotoxic functions., and its dysregulation can lead to abnormal maturation and function of these innate immune cells.<sup>57</sup> By influencing the behavior of these vital immune cell types, dysregulated expression of miRNAs like miR-210 and miR-150 may drive immunological dysfunction and inflammation-related tissue damage in thalassemia patients.<sup>58-60</sup> Moreover, studies have demonstrated that miR-150 can govern T cell activation and development, whereas miR-21 modulates the generation of potent cytokines including IL-6 and TNF- $\alpha$ .<sup>61</sup> The miR-21 in macrophages promotes an anti-inflammatory M2 phenotype polarization by targeting PDCD4, a negative regulator of IL-10.<sup>62</sup> In addition to effects on immune cells, research indicates that miRNAs can influence the output of cytokines and chemokines, key inflammatory mediators implicated in thalassemia pathogenesis. Several studies have also demonstrated that miR-let-7b, miR-155, and miR-146a control inflammation in thalassemia by regulating the NF- $\kappa$ B pathway, a central coordinator of immune responses. In thalassemia, downregulation of let-7b miRNA family members (let-7b-5p, let-7i-5p) is predicted to impact signaling pathways like TGF- $\beta$ , PI3K/AKT, FoxO, Hoppo, and MAPK, which are crucial for immune cells development and function.<sup>63</sup> By modulating this critical signaling cascade, as well as downstream cytokine and chemokine production, altered expression of these miRNAs may drive aberrant inflammation and tissue damage in patients with thalassemia.<sup>64-66</sup>

Moreover, literature indicates that miR-150 and miR-155 are integral to modulating immune reactions associated with transfusions in thalassemia. These miRNAs influence B cell maturation, T cell activation, and immunoglobulin production in frequently transfused patients with thalassemia.<sup>50,67</sup> Furthermore, miR-150 overexpression in thalassemia can disrupt B cell development, resulting in a reduced population of mature B cells and potentially compromising antibody production and immune responses to transfused blood components.<sup>63,68</sup> A study showed that miR-150

deficiency in mice resulted in increased numbers of B-1 cells and elevated levels of natural antibodies, which could contribute to transfusion reactions.<sup>69</sup> The elevated level of miR-155 is stored to enhanced expression of interferon-gamma (IFN- $\gamma$ ) and interferon beta (IFN- $\beta$ ), and its deregulation can lead to uncontrolled immune cell proliferation and activation of inflammatory pathways.<sup>70</sup> The miR-155 is upregulated upon T cell activation and plays a crucial role in regulating T cell proliferation, differentiation, and cytokine production,<sup>4</sup> in patients with leukemia and thalassemia who have aberrant T-cell activation and an exaggerated inflammatory response, potentially contributing to a transfusion-related immune reaction.<sup>71</sup> The miR-150 and miR-155 have also been implicated in regulating immunoglobulin production by B cells, targeting the transcription factor c-Myb, which is essential for B cell development, immunoglobulin gene recombination, and activation-induced cytidine deaminase (AID) as well as Blimp-1, both involved in class-switch recombination and plasma cell differentiation. It may lead to altered immunoglobulin production and contribute to transfusion-related immune reactions.

### **miRNAs and oxidative stress**

The oxidative stress in thalassemia is amplified by the presence of free iron radicals, heme groups, and non-heme iron. These reactive species are generated due to the disrupted balance in globin chain production, leading to an excess of unbound iron contributing to the higher oxidative state. All of these stimulate the production of excessive free radicals. Moreover, levels of antioxidant proteins are reduced in thalassemic erythrocytes. Increased levels of oxidative stress trigger a cascade of detrimental effects, including accelerated RBC destruction, impaired erythropoiesis, and dysfunction of critical organs like the heart and liver.<sup>65,72</sup>

The miRNAs have also a significant impact on oxidative stress in patients with thalassemia. Previous research has demonstrated that elevated levels of miR-144 expression correlate with a markedly heightened susceptibility to the destructive effects of oxidative stress. The downregulation of miR-144 level was also linked to elevated levels of the nuclear factor erythroid 2-related factor 2 (NRF2) and glutathione, especially in  $\alpha$ -thalassemia. Aberrant expression levels of miR-144 could potentially contribute to the impaired capacity of RBCs to counteract oxidative stress, consequently increasing their vulnerability to premature destruction through hemolysis.<sup>73</sup>

Another study reported that oxidative stress has the potential to induce miR-210 and miR-214 upregulation in thalassemia. The potential involvement of miR-210 in oxidative stress-induced myocardial injury has been also explored. The miR-210 exerts a shielding effect against hydrogen peroxide-triggered apoptosis in cardiomyocytes by modulating the BNIP3expression, a pro-apoptotic protein that interacts with the Bcl-2 adenovirus E1B

19kDa protein.<sup>74</sup> The miR-214 was also found associated with higher levels of reactive oxygen species (ROS), while an inverse relationship was observed between miR-214 expression and erythrocyte index including MCV, MCH, and MCHC. During Hemoglobin H disease, the higher levels of miR-214 can be detected. However, no significant difference in miR-214 expression was observed in the immature RBCs HbE/thalassemia. However, a decrease in the activating transcription factor 4 (ATF4) target was reported in both thalassemic groups. The findings suggested that the elevated expression of miR-214 was associated with both increased oxidative stress and the severity of anemia in patients with Hemoglobin H disease. This correlation was attributed to the suppression of the ATF4 by miR-214.<sup>75</sup>

Another study revealed that increased miR-92a-3p levels led to elevated  $\gamma$ -globin, glutathione, and superoxide dismutase expression, while reducing ROS, malondialdehyde, and cell apoptosis. Significantly, BCL11A, a regulator of hemoglobin synthesis, was recognized as a direct target of miR-92a-3p, downregulated by this microRNA.<sup>76</sup> Regarding the activation and quantification of ROS in thalassemia patients, it is known that miR-9 in erythroid precursor controls the expression level of FoxO3, Btg1, and Cited 2. However, FoxO3-3A prevents the inhibition of erythroid cell maturation and ROS levels caused by miR-9.<sup>77</sup>

#### ***miRNAs and iron metabolism***

Given that miRNAs are essential in regulating iron metabolism by targeting iron uptake, storage, and utilization genes, available evidence highlighted the miRNAs key role toward iron metabolism in patients with thalassemia. For instance, miR-122 has been shown to regulate iron metabolism by targeting hepcidin, a key regulator of iron homeostasis.<sup>78</sup> Specific miRNAs have been demonstrated to regulate key proteins involved in iron homeostasis. In addition, miR-485-3p has the potential to regulate the expression of transferrin receptor 1 (TfR1), which facilitates cellular iron uptake. On the other hand, elevated levels of miR-485-3p suppress TfR1 expression, leading to reduced iron acquisition. In contrast, upregulated miR-20a can diminish the ferritin levels, a protein that is essential for intracellular iron storing, and further promoting iron release.<sup>2</sup> Through modulating the expression of these critical iron handling proteins, miRNAs like miR-485-3p and miR-20a act as integral regulators of systemic and cellular iron balance. Dysregulation of these iron-related miRNAs may contribute to the iron overload observed in patients with thalassemia.<sup>79</sup>

In addition to miR-485-3p and miR-20a, other miRNAs like miR-210, miR-122, and let-7b have emerged as regulators of iron homeostasis. Also, miR-210 is increased under low oxygen and modulates several iron-related genes including TfR1, ferroportin, and hepcidin.<sup>1</sup> Of

note, Hepcidin, a hormone that inhibits iron absorption and recycling, is also regulated by miR-122. In turn, miR-let-7b regulate ferroportin, a key iron export protein. Therefore, the miRNAs comprise an iron-regulatory network through targeting critical proteins involved in systemic and cellular iron handling.<sup>79</sup>

Various factors can disrupt miRNAs regulating iron metabolism, including genetic mutations, environmental exposures, and disease states. Mutations in miRNA genes or their targets can alter miRNA expression and function. Toxins and pollutants also influence miRNA activity. Ultimately, the disease can dysregulate iron-related miRNAs like miR-20a, miR-451, miR-146a, and miR-155, contributing to anemia, inflammation, and iron overload.<sup>36,80</sup>

It is worth noting that MiR-485-3p dysregulation has a crucial role in the development of iron deficiency anemia. MiR-122 is also critical, regulating hepcidin to control iron absorption and recycling. With iron deficiency, miR-122 is also downregulated, increasing hepcidin and reducing iron uptake, respectively. In addition, MiR-122 modulates other iron genes like TfR1, ferroportin, and HO-1. By regulating multiple effectors in iron metabolism, disruption of key miRNAs like miR-485-3p and miR-122 may profoundly alter iron homeostasis in thalassemia.<sup>52</sup> Additional miRNAs involved in iron metabolism and deficiency include miR-let-7b, miR-20a, miR-200b, and miR-185. These miRNAs modulate genes involved in iron uptake, storage, and consumption. For example, miR-let-7b and miR-20a regulate ferroportin expression, and ferritin serum levels, respectively. Through effects on key iron handling proteins, disruption of these regulatory miRNAs may alter cellular and systemic iron equilibrium.<sup>81</sup>

#### ***miRNAs and cardiac function***

The major complication observed in thalassemic individuals refers to the development of congestive heart failure (HF) due to regular blood transfusions, leading to biventricular systolic dysfunction, and early mortality due to cardiomyopathy.<sup>82-84</sup>

Existing data from sequencing projects in the healthy adult heart has identified a subset of miRNAs substantially upregulated in normal cardiomyocytes. Several miRNAs, such as miR-1, coupled with miR-16 and miR-27b, have been implicated in preserving cardiac health. However, miR-30d, accompanied by miR-126, miR-133, miR-143, and miR-208, as well as the let-7 family contributing to cardiovascular diseases (CVD). Consequently, these miRNAs may hold a pivotal role in preserving normal cardiac function and in the development of CVD.<sup>85</sup>

The abundance of miR-765 leads to diminished levels of inhibitor-1, consequently impairing the contractile ability of cardiomyocytes and modulation of calcium dynamics via the protein phosphatase 1 (PP-1) signaling pathway. Down-regulation of miR-765 may serve as an

additional therapeutic target to inhibit the increased PP-1 activity and improve calcium handling in failing hearts.<sup>86</sup> Also, it has been found that the miR-155 is upregulated in patients with HF and positively correlates with left ventricular mass index, which is a prognostic marker for HF. Previous studies, using transgenic animal models, have demonstrated the clinical relevance of miR-155 involvement in HF. Importantly, knockout of miR-155 was shown to improve cardiac remodeling, potentially through targeting the tumor protein p53-inducible nuclear protein 1 (TP53INP1).<sup>87</sup>

The studies in animal models propose that anti-miR-212 may be favorable agents for the enhance the cells remodeling during HF. It is also the same with anti-miR-132 and anti-miR-562 agents. Despite the potential effects, several challenges and drawbacks associated with miRNA-based therapeutic approaches have been recognized. As an illustration, the therapeutic efficacy of anti-miR-34a has exhibited a gender-based discrepancy, demonstrating superior outcomes in females with moderate dilated cardiomyopathy compared with male counterparts.<sup>88,89</sup>

Moreover, microRNA-21 has the potential to modulate pro-fibrotic signaling toward cardiac fibroblasts and thus manage cardiac restoration. Quantitative small RNA sequencing analysis of purified myocardial cell populations also identified the highest levels of miR-21 expression within cardiomyocytes.<sup>90</sup>

Important functional interactions between myocardial infarction-associated transcript (MIAT), and miR-150, represent new regulatory mechanisms related to ischemic HF, including the Hoxa4 factor. In a recent study, it has been found that overexpressing miR-150 can alleviate the detrimental effects caused by the MIAT after a heart attack. Through genome-wide analysis of MIAT-deficient mouse hearts, they identified Hoxa4 as a novel downstream target of the MIAT/miR-150 axis. Notably, Hoxa4 was also found to be upregulated in patients with HF, underscoring the potential clinical significance of this molecular pathway.<sup>91</sup> MicroRNA-25 was also enriched in early-stage human embryonic stem cell-originated cardiac progenitors, however, its levels steadily declined over time. Increasing the levels of miR-25 facilitated the multiplication of cardiomyocytes by downregulating the expression of F-box and WD repeat domain containing 7 (FBXW7), a gene involved in regulating cell division. Zebrafish studies have also revealed the function of miR-25 in the proliferation of cardiomyocytes, making the miR-25 as a potential molecule in cardiomyocytes rejuvenation.<sup>92</sup> Considering the positive role of some miRNAs, such as miR-30d cardioprotective effect against HF, and they may be new therapeutic targets in this era.<sup>93</sup>

Exosome-mediated modalities have been considered strikingly optimistic strategy for cardiac function regeneration. Histological examinations revealed that in rat models with myocardial infarction (MI) subjected to

treatment with alginate hydrogel vehicles encapsulating exosomes overexpressing miR-126 and miR-146a mimics exhibited diminished levels of fibrosis coupled with elevated deposition of collagen fibers. The results underscore a therapeutic potential for the treatment of MI through the regulation of angiogenesis and vascular integrity.<sup>94</sup>

### ***miRNAs and liver function***

Hepatic complications in patients with thalassemia due to iron overload include inflammation, fibrosis, and finally cirrhosis. Hepatic T2\*, a non-invasive technique is utilized to evaluate the excessive accumulation of iron in the liver and heart, enabling early detection and intervention before the manifestation of overt symptoms or complications associated with iron overload.<sup>95</sup> Liver cirrhosis is regarded as the final pathological outcome of various chronic liver diseases, with fibrosis being considered its precursor. The development of fibrotic and cirrhotic liver involves various cytokines that regulate inflammatory responses, cell proliferation, and tissue remodeling processes. While specific miRNAs modulate gene expression, influencing stellate cell activation, and matrix production. The hepatic stellate cells activation is viewed as an essential event in the development of fibrosis and is considered a crucial event during the fibrotic process.<sup>96</sup>

MicroRNA-122, a microRNA species exhibiting pronounced hepatic enrichment, has been the subject of extensive investigations delving into its biological roles and clinical relevance. It fulfills critical functions in regulating hepatic processes and has also been implicated in liver pathogenesis. Although in patients with liver fibrosis, miR-122 is downregulated, it has been shown that it is upregulated in patients with liver cirrhosis. Individuals with advanced cirrhotic liver disease exhibited a marked reduction in their serum miR-29a concentrations. Serum levels of miR-138 and miR-143 have been characterized as being indicative of the later stages of liver fibrosis, thus in early stages this marker may not be effective in the diagnosis molecules.<sup>97</sup>

Stem cells derived from adipose tissue are also regarded as a conveniently accessible source of multipotent progenitor cells with the ability to differentiate into various mesenchymal lineages. These cells have emerged as a promising therapeutic option in the field of regenerative medicine. Following 70% partial hepatectomy in rats, ASCs-miR-27b was administered. This intervention led to decreased levels of cytokines associated with inflammatory responses, upregulated hepatocyte, and other growth factors. ASCs-miR-27b administration has been shown to modulate inflammatory responses and promote liver regeneration after partial hepatectomy.<sup>98</sup>

Experimental studies demonstrated that the miR-378 has emerged as a key modulator of non-alcoholic steatohepatitis (NASH) via tumor necrosis factor (TNF)  $\alpha$  signaling. In this regard, miR-378 is an integral

part of the complex molecular pathways to induce unprompted induction of inflammatory genes, which may have important implications for the development and progression of NASH. Therefore, understanding the physiological roles of miR-378 in regulating hepatic inflammation and liver injury is important to address the complexity of the development of NASH and fibrosis.<sup>99</sup>

MicroRNA 27b inhibits ubiquitin-specific peptidase 4 (USP4), an enzyme that targets TGF- $\beta$  receptor 1. It has been also recognized that liver X receptor alpha (LXR $\alpha$ ) was found to protect liver cells from adverse stimuli and attenuate liver damage and fibrotic process. The liver X receptor confers a protective effect on hepatocytes against the detrimental impact of TGF- $\beta$  through transcriptional regulation of the CB2 gene, leading to the inhibition of USP4 activity by the CB2 receptor, which stabilizes T $\beta$ RI. The inhibitory role of miR-27b on USP4, a deubiquitylating enzyme for T $\beta$ RI, was identified downstream of the CB2 signaling pathway.<sup>100</sup> An intravenous delivery of miR-23b/27b/24-1 lentivirus, increasing the levels of the miR-23b/27b/24-1 cluster, was found to ameliorate hepatic fibrosis in mice. This cluster exhibited an inhibitory effect on the development of hepatic fibrosis, indicating its potential as an innovative therapeutic approach for individuals suffering from this condition.<sup>101</sup>

#### ***The miRNAs modulation: as a promising option for Thalassemia Treatment***

miRNAs modulation could be achieved via strategies such as stimulators and inhibitors. The miRNA mimics are synthetic RNAs designed to imitate endogenous miRNAs. While miRNA inhibitors are antisense oligonucleotides that can bind and suppress specific miRNAs.<sup>102</sup> By altering miRNA levels, these approaches may potentially restore balanced gene expression in thalassemic cells and improve symptoms.

Several studies have examined miRNA modulation effects on thalassemia in preclinical and clinical settings. It has been shown that miR-27a overexpression could increase fetal hemoglobin production and reduce anemia in a mouse model.<sup>103</sup> Also, miR-144 inhibition could increase fetal hemoglobin and improve erythropoiesis. Overall, miRNA modulation is a promising therapy warranting more research. Targeting relevant miRNAs could reinstate normal gene patterns and alleviate symptoms. However, further preclinical and clinical studies are required to fully evaluate safety and efficacy.

Previous literature indicated that upregulation of miR-15a and miR-16-1 may enhance erythropoiesis in  $\beta$ -thalassemia patients.<sup>4,24</sup> Similarly, increased miR-221 and miR-222 could reduce erythroid apoptosis in  $\alpha$ -thalassemia. A study of high fetal hemoglobin individuals found that the expression of miR-15a and miR-486-3p was significantly different when compared with the control group. Other miRNAs, including miR-16-1 and miR-96, also exhibit similar expression

patterns, suggesting their potential involvement in fetal hemoglobin regulation. Another study also revealed that miR-486-3p and miR-15a play significant roles in fetal hemoglobin production, together with microRNA-486-3p specifically targeting and downregulating BCL11A, a transcriptional repressor of  $\gamma$ -globin gene expression. The miR-15a and miR-16-1 directly inhibit MYB, a hemoglobin gene silencer. Thus, both miRNAs may have an influence on fetal hemoglobin expression and could serve as therapeutic targets.<sup>104</sup>

#### **Limitations**

While this insight delivers a comprehensive overview of the roles of miRNAs in thalassemia pathophysiology, it is important to acknowledge certain limitations. A major challenge is the efficient delivery of miRNA mimics/inhibitors to target cells and tissues. Many studies use intravenous injection, which may not effectively reach the bone marrow.<sup>103</sup> Systemic delivery risks off-target effects and toxicity. Animal models may also not fully reflect human thalassemia complexity.<sup>105,106</sup> Many studies use single miRNAs, which does not address the multifaceted mechanisms of thalassemia.<sup>106-108</sup> Additionally, the lack of standardized miRNA measurement impedes result comparisons and translation.<sup>107</sup> More extensive clinical trials are needed to evaluate the safety and efficacy of miRNA modulation strategies.

Secondly, the studies included in this review are pre-clinical investigations, conducted either *in vitro* or *in vivo*. Translating these findings to human clinical settings may pose challenges due to the inherent complexity of thalassemia disorders and the potential differences in miRNA expression and regulation between model systems and human patients. Third, most studies have focused on examining the effects of individual miRNAs or a limited number of miRNAs. However, miRNAs operate within intricate regulatory networks, and their functions may be influenced by the interplay with other miRNAs, as well as other regulatory molecules such as long non-coding RNAs and transcription factors. Investigating the combined effects of multiple miRNAs and their interactions with other regulatory elements may provide a more comprehensive understanding of their roles in thalassemia. Additionally, the methodologies used for miRNA profiling and quantification vary across studies, which can lead to inconsistencies in the reported miRNA expression levels and functional implications. Standardization of miRNA analysis techniques and data normalization approaches is crucial for ensuring the reproducibility and comparability of results.

#### **Conclusion**

This review underscores the substantial role of miRNAs in thalassemia, elucidating their regulatory mechanisms, biomarker potential, and therapeutic promise. MicroRNAs intricately modulate gene expression, impacting critical

processes including erythropoiesis, iron metabolism, apoptosis, and inflammation—all central to thalassemia pathogenesis. Understanding this complex regulatory network presents exciting therapeutic targets and emphasizes the clinical utility of miRNAs as diagnostic, prognostic, and treatment response biomarkers.

## Future Directions

Upon the findings presented in this review, some future directions can be considered to better understanding of miRNAs in thalassemia and translate this knowledge into clinical settings:

1. Performing large-scale clinical studies to validate the promising of miRNAs as diagnostic and prognostic molecules in thalassemia patients. These studies should aim to establish standardized protocols for miRNA quantification and normalization, ensuring the reliability and reproducibility of results across different clinical settings.
2. Exploring the feasibility of miRNA-based therapeutic approaches through pre-clinical and clinical trials. This may involve developing efficient delivery systems for miRNA mimics or inhibitors, evaluating their safety and efficacy, and investigating potential combinatorial strategies with existing therapies.
3. Integrating multi-omics data, including transcriptomics, proteomics, and metabolomics, to gain a more comprehensive understanding of the regulatory networks involving miRNAs and their functional implications in thalassemia pathogenesis.
4. Investigating the potential of miRNA-based personalized medicine approaches, where individualized miRNA profiles could be used to tailor treatment strategies and monitor therapeutic responses in thalassemia patients.
5. Exploring the potential of miRNAs as targets for gene editing technologies, such as CRISPR/Cas9, to modulate their expression and function in thalassemia disorders.
6. Designing longitudinal studies to assess the dynamic changes in miRNA expression profiles throughout thalassemia progression and in response to various therapeutic interventions.

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## Authors' Contribution

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## Study Highlights

### What is current knowledge?

- MicroRNAs (miRNAs) play a critical role in the regulation of gene expression in thalassemia, particularly in erythropoiesis, iron metabolism, immune responses, and oxidative stress. They are known as promising biomarkers for diagnosing transfusion-related complications and as potential therapeutic targets to modulate disease mechanisms.

### What is new here?

- This review provides a comprehensive synthesis of recent advancements in miRNA research related to thalassemia. It highlights specific miRNAs like miR-16, miR-210, and miR-451a as pivotal players in erythropoiesis and iron metabolism. The review also discusses emerging miRNA modulation strategies, including mimics and inhibitors, that could pave the way for innovative therapeutic approaches tailored to individual patient needs.

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## Competing Interests

The authors state that there are no conflicts of interest regarding the publication of this manuscript. The authors have made substantial contributions to the work and agree with the manuscript's results and conclusions. This manuscript has been read and approved by all named authors, and there are no relationships or activities that have influenced or biased the work.

## Ethical Approval

Not applicable.

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