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Original Article

Effects of Thalidomide on Erythropoiesis and Iron Homeostasis in Transfusion-Dependent β-Thalassemia

Kun Yang*, Xiaodong Liu, Wei Peng, Fang Hua, Lan Li, Kun Chen, Jin Zhang, Shan Luo, Wanting Li, Yuxi Ding, Jie Chen and Jian Xiao*.

Department of Hematology, Zigong First People's Hospital, Zigong, China.
* The authors equally contributed to the work.

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Abstract. Thalidomide is a therapeutic option for patients with β-thalassemia by increasing fetal hemoglobin and thereby reducing the requirement for blood transfusions. However, information on changes in erythropoiesis and iron homeostasis during thalidomide treatment is lacking. This study investigated the effects of thalidomide treatment on hematologic, erythropoietic, and iron-status parameters in 22 patients with transfusion-dependent β-thalassemia (TDT). Thalidomide significantly improved anemia endpoints, including increases in hemoglobin (p<0.001), red blood cells (p<0.001), and hematocrit (p<0.001), as well as reducing erythropoietin levels (p=0.033) and ameliorating erythropoiesis. Thalidomide treatment significantly reduced serum iron levels (p=0.018) and transferrin saturation (p=0.039) and increased serum transferrin levels (p=0.030). Thalidomide had no observed effect on serum ferritin or hepcidin, but changes in hepcidin (r=0.439, p=0.041) and serum iron (r=−0.536, p=0.010) were significantly correlated with hemoglobin increment. This comprehensive study indicates that thalidomide treatment can ameliorate erythropoiesis and iron homeostasis in patients with TDT, thus supporting the effectiveness of this drug.

Keywords: Thalidomide, β-thalassemia, Erythropoiesis, Iron homeostasis.

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Correspondence to: Kun Yang. Department of Hematology, Zigong First People's Hospital, Zigong, China. E-mail: 1759874951@qq.com
Jian Xiao. Department of Hematology, Zigong First People's Hospital, Zigong, China. E-mail: 16188702@qq.com

Introduction. Thalassemia is an inherited blood disorder affecting the synthesis of globin chains.[1] The severity of anemia, need for transfusions, and clinical morbidity of β-thalassemia are closely tied to the degree of imbalance between α-globin and β-globin chains. Deficiency of β-globin chains leads to the accumulation of excessive, unstable α-globin tetramers in erythrocytes. Free α-globin is unstable, produces cytotoxic active oxidants and cell pellets, impairs the maturation and viability of erythrocyte precursors, and leads to ineffective erythropoiesis (IE) and premature hemolysis of circulating erythrocytes, resulting in anemia and decreased erythrocyte survival.[2] Increased erythropoietin (EPO) due to chronic anemia further increases IE, bone marrow dilation, and extramedullary hematopoiesis.[3] Disordered iron homeostasis is a central feature of the pathophysiology of thalassemia. In transfusion-dependent β-thalassemia (TDT) patients, iron intake saturates serum transferrin, leading to non-transferrin-bound iron species that accumulate in tissues and cause

damage to vital organs.[4]

Re-expression of γ-globin and more efficient synthesis of fetal hemoglobin (HbF) can reduce the imbalance between α-globin and β-globin chains, and induction of HbF has been used as a treatment strategy for β-thalassemia.[5] Thalidomide and its derivatives are used to treat some malignant hematologic diseases because of their anti-inflammatory, anti-tumor, anti-neovascularization, and immunomodulatory properties.[6] Thalidomide can also induce expression of the γ-globin gene, which increases HbF levels.[7] Previous studies demonstrated significant efficacy of thalidomide in patients with TDT or non-transfusion-dependent β-thalassemia (NTDT);[8-10] however, information on changes in erythropoiesis and iron homeostasis during thalidomide treatment is lacking.

In this study, we evaluated the effects of thalidomide on erythropoiesis and iron homeostasis and analyzed the correlations between baseline indicators and hemoglobin changes to explore the possible mechanisms of thalidomide in the treatment of β-thalassemia and the possibility of combining thalidomide with other agents.

Methods

Patients. This study included TDT patients treated with thalidomide for >3 months in Zigong First People's Hospital. TDT was diagnosed according to the Thalassemia International Federation guidelines.[4] The inclusion criteria were: 1) age 14–18 years; 2) diagnosis of TDT using accepted clinical and genetic methods; and 3) ECOG physical score 0–2 points. The exclusion criteria were: 1) therapy with drugs that might affect Hb levels 3 months before enrolment; 2) other hemolytic disorders; 3) cardiopulmonary, cerebrovascular, liver, kidney, or other severe diseases; 4) allergy to thalidomide; and 5) currently participating in any other clinical trial. Patients were informed of the side effects and possible benefits of thalidomide. All patients were warned against becoming pregnant or impregnating a woman while taking the drug. The study protocol was approved by the ethics committee of Zigong First People's Hospital. The study adhered to the principles of the Declaration of Helsinki, and written informed consent was obtained from all participants and their guardians.

Treatment. Thalidomide (Changzhou Pharmaceutical Factory, Changzhou, Jiangsu, China) was administered at 100 mg/day. Transfusion was recommended to maintain hemoglobin levels >9.0 g/dL during treatment, and regular transfusion volumes were administered if hemoglobin fell below this level. Aspirin was prescribed to patients with platelet counts $>500\times10^9$/L to prevent thrombosis. These patients did not receive iron chelation therapy during the first trimester of thalidomide treatment.

Laboratory examinations. Venous blood samples were collected before thalidomide and after 3 months of treatment, respectively, and before transfusion. Complete blood counts were analyzed using an XE 5000 automatic blood cell analyzer (Sysmex Corporation, Kobe, Japan). Hb levels were quantified by high-pressure liquid chromatography (Bio-Rad Variant II, Bio-Rad, Hercules, CA, USA). Biochemical parameters were assessed using a multichannel analyzer (Abbot Aeroset, Abbott Diagnostics, Bohemia, NY, USA). Samples were tested for serum iron, total iron-binding capacity (TIBC), unsaturated iron-binding capacity (UIBC), and transferrin saturation by the colorimetric method (Pointe Scientific, Inc., Canton, MI, USA), serum ferritin by immunoassay (Immulite 1000), and soluble transferrin receptor (sTfR), EPO (R&D Systems), and hepcidin (Intrinsic Life Sciences, La Jolla, CA, USA) by enzyme-linked immunosorbent assay.

Statistical analysis. Data were analyzed using SPSS Statistics 26.0 (SPSS Inc., Chicago, IL, USA). Numerical data were presented as mean ± standard deviation or median and interquartile range. Changes in continuous variables before and after treatment were compared by paired *t*-test or Mann–Wilcoxon rank-sum test. Correlations were analyzed by linear regression and univariate analysis. A p-value <0.05 was considered significant in all analyses.

Results

Patient characteristics. Twenty-two patients were included in this study between May 2021 and August 2022. The patient cohort comprised 14 males and eight females, with a median age of 15 years (range: 14–18 years). Splenectomy was performed in 18.2% (4/22) of the patients.

Effects of thalidomide treatment on hematologic and erythropoietic parameters in TDT patients. Hematologic parameters improved after thalidomide treatment compared with baseline. Specifically, thalidomide treatment significantly improved anemia endpoints, including increased hemoglobin (p<0.001), red blood cells (RBCs) (p<0.001), and hematocrit (p<0.001) (**Figure 1A-D**). Thalidomide reduced the mean corpuscular volume (MCV) (p<0.001) and mean erythrocyte hemoglobin (MCH) (p=0.011) (Figure 1E-G), and reduced lactate dehydrogenase, suggesting that thalidomide treatment reduced hemolysis (**Figure 1H-K**). Thalidomide also significantly reduced EPO, further demonstrating an improvement in anemia after treatment (**Figure 1L**). The detailed data are shown in **Supplementary Table S1**.

Effects of thalidomide treatment on serum iron-status

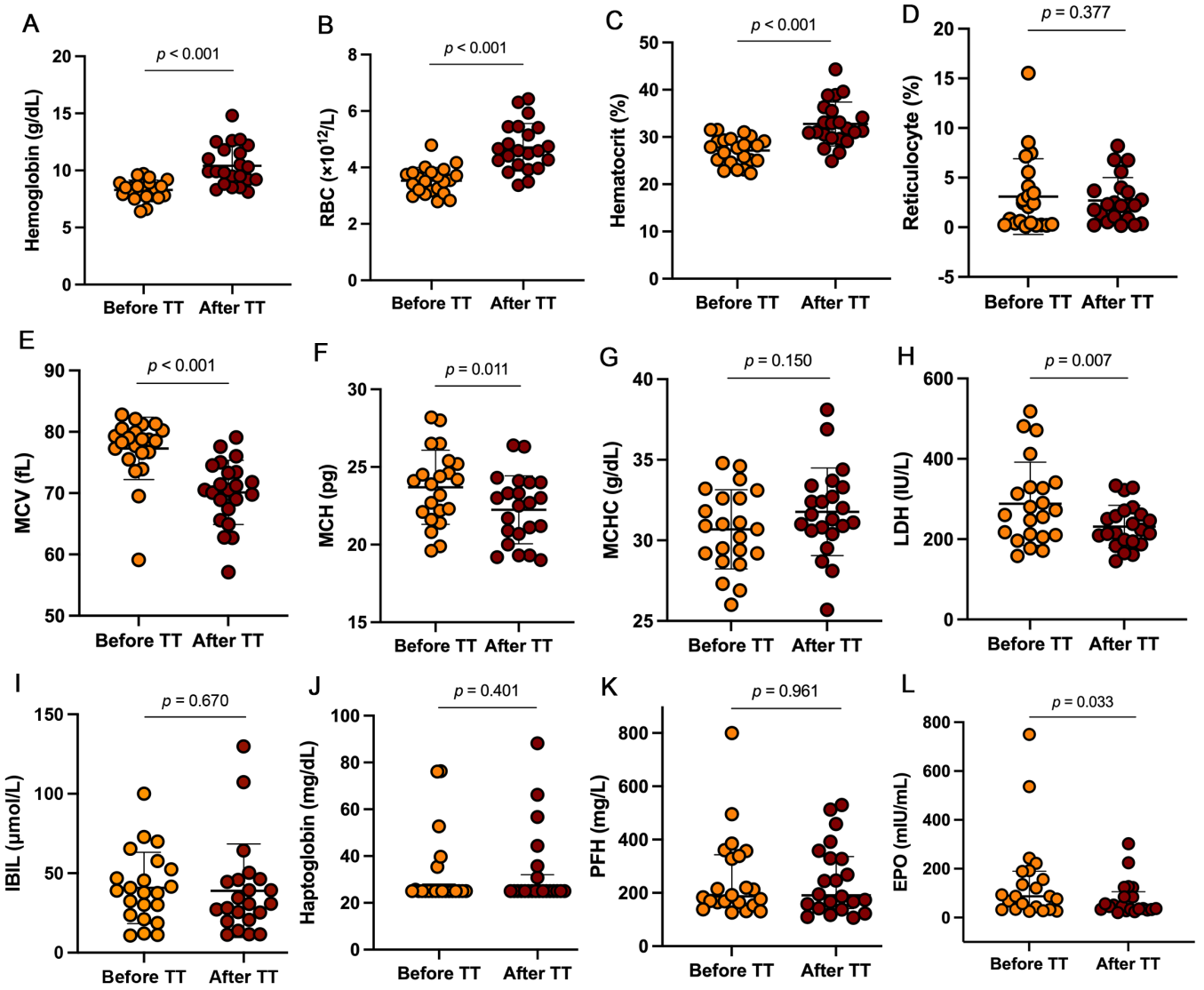

Figure 1. Thalidomide treatment (TT) affected hematologic and erythropoietic parameters in TDT patients. (**A**) Hemoglobin levels, (**B**) red blood cell (RBC) count, (**C**) hematocrit, (**D**) reticulocytes, (**E**) mean corpuscular volume (MCV), (**F**) mean corpuscular hemoglobin (MCH), and (**G**) mean corpuscular hemoglobin concentration evaluated 3 months after TT showed anemia improvement. (**H-K**) Hemolysis parameters, including lactate dehydrogenase (LDH), were significantly decreased, but indirect bilirubin (IBIL), haptoglobin, and plasma free hemoglobin (PFH) were unchanged. (**L**) Reduced erythropoietin levels indicated ameliorated erythropoiesis.

parameters in TDT patients. Thalidomide treatment significantly reduced serum iron levels (p=0.018) (**Figure 2A**) and transferrin saturation (p=0.039) (**Figure 2B**). Moreover, serum transferrin levels increased after thalidomide treatment (p=0.030) (**Figure 2C**). There was no significant change in serum ferritin, hepcidin, sTfR, TIBC, or UIBC (**Figure 2D-H**). The detailed data are shown in **Supplementary Table S2**.

Correlation between changes in erythropoiesis and iron-status parameters and hemoglobin increment. We investigated the correlations between changes in erythropoiesis and iron-status-related parameters and prolonged hemoglobin increment after thalidomide treatment. Hemoglobin increment was significantly correlated with changes in RBCs (r=0.839, p<0.001), hematocrit (r=0.813, p<0.001) and hepcidin (r=0.439, p=0.041) (**Figure 3A-C**). In addition, hemoglobin increment was negatively correlated with serum iron (r=−0.536, p=0.010) (**Figure 3D**). There were no correlations between changes in other parameters and hemoglobin increment after thalidomide treatment (**Supplementary Table S3**).

Discussion. Thalidomide has recently become a treatment option for patients with β-thalassemia, under strict medical supervision or in clinical trials, especially for patients who are unable to undergo hematopoietic stem cell transplantation. Thalidomide has shown promise for increasing HbF and reducing the need for transfusions in patients with β-thalassemia.[9-11] In addition, thalidomide significantly reduced spleen size and may be used to treat thrombocytopenia in patients with hypersplenism.[12,13] Chen et al.[13] also observed that

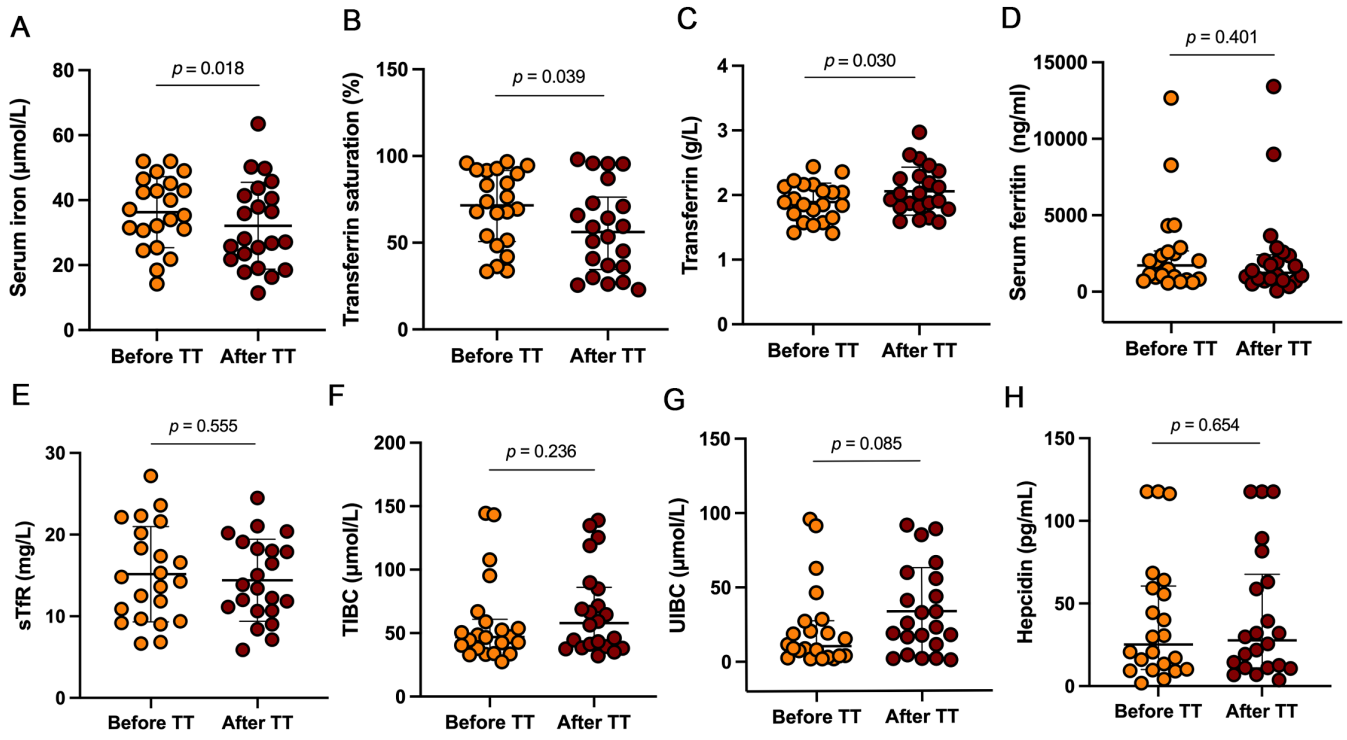

Figure 2. Thalidomide treatment (TT) affected serum iron-status parameters in TDT patients. (**A**) Serum iron and (**B**) transferrin saturation were significantly decreased, and (**C**) transferrin was significantly increased, (**D-H**) but serum ferritin, hepcidin, soluble transferrin receptor (sTfR), total iron-binding capacity (UIBC), and unsaturated iron-binding capacity (UIBC) were unaffected.

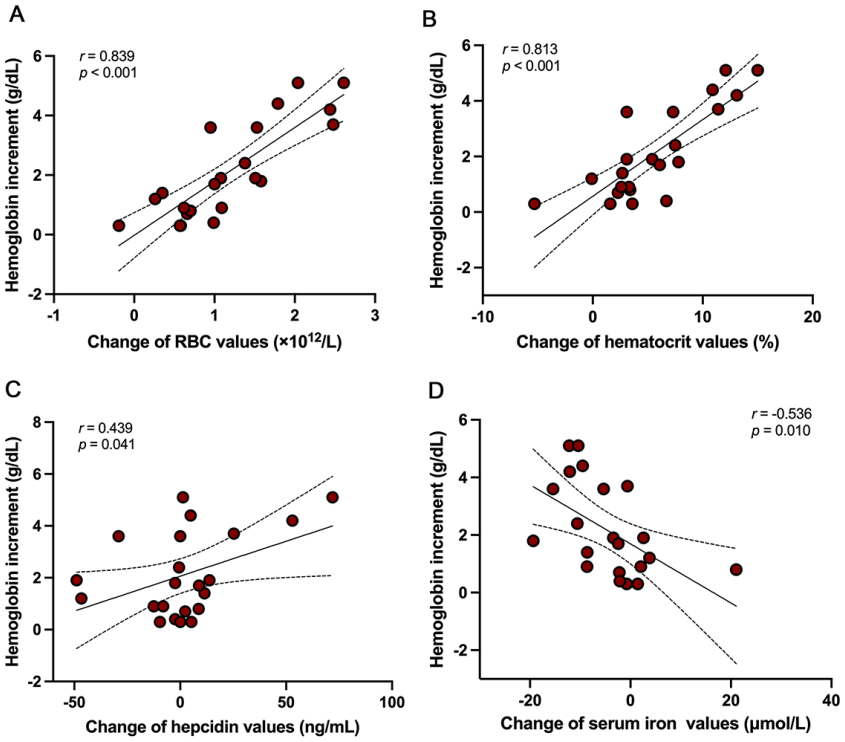

Figure 3. Plots of prolonged hemoglobin increment after thalidomide treatment versus changes in (**A**) red blood cells (RBCs), (**B**) hematocrit, (**C**) hepcidin, and (**D**) serum iron.

thalidomide improved organ iron deposition in patients with TDT. The current study found that thalidomide increased the concentration of circulating RBCs and Hb, reduced serum EPO levels, reduced serum iron and transferrin saturation, and increased transferrin levels in patients with TDT. To the best of our knowledge, this study offers the first comprehensive analysis of erythropoiesis and iron homeostasis in patients with

thalassemia, providing evidence to support the use of thalidomide for treating anemia in thalassemia.

After thalidomide treatment, RBCs increased while EPO levels and reticulocytes were reduced. Thalidomide thus appeared to increase RBC production and maturation in patients with TDT, resulting in more effective erythropoiesis. The decreased serum EPO concentration may be due to feedback regulation by the increased number of circulating RBCs and increased Hb concentration. In addition, improved erythropoiesis may be associated with decreased formation of insoluble globins (α chain/heme aggregates). Thalidomide acts as an HbF inducer to enhance the expression of γ-globin, which binds to redundant α-globin chains and reduces the deposition of α-globin chain tetramers, thereby reducing their potential toxicity when adhering to erythrocyte membranes and producing reactive oxygen species.[15] The extramedullary hematopoiesis rate increases in patients with β-thalassemia to compensate for anemia, resulting in increased production and clearance of abnormal RBCs, with hypersplenism and increased spleen size.[16] Chen et al.[13] reported a progressive decrease in spleen length in thalassemia patients treated with thalidomide at 12 months of follow-up, which may also indicate improved erythropoiesis.

Blood transfusion can halt disease progression by providing normal RBCs, inhibiting the production of ineffective RBCs, and reducing extramedullary hematopoiesis. However, repeated blood transfusions can lead to iron accumulation and overload. Theoretically, thalidomide treatment will relieve the body's iron burden by reducing blood transfusions in patients with severe thalassemia. However, although serum ferritin levels decreased following thalidomide treatment in the present study, the difference was not significant. This may be because changes in hepatic iron deposition after thalidomide treatment are inconsistent with changes in serum ferritin levels over time. Chen et al.[14] observed a significant decrease in serum ferritin levels up to 12 months after thalidomide treatment but significant reductions in hepatic iron deposition at 3 and 12 months of treatment, suggesting that hepatic iron deposits may be reduced during thalidomide therapy even when serum ferritin levels are not significantly changed. Thalidomide tended to reduce transferrin saturation and serum iron levels after treatment, possibly due to increased iron consumption due to enhanced erythropoiesis. When transferrin saturation is reduced, the predominant form of transferrin in circulation is monoferric-transferrin, whose each molecule delivers less iron to erythroid precursors than holo-transferrin.[17] This enables more erythroid precursors to receive a smaller portion of the iron pool to offset developing anemia and is consistent with a low MCV and MCH. Therefore, Thalidomide treatment results in a state of iron-restricted-like erythropoiesis. Typically, as in iron-deficiency anemia, iron-restricted-like erythropoiesis is associated with low MCV and MCH values, where the amounts of heme and Hb per cell are lower due to the delivery of less iron to each RBC precursor and the production of fewer cells, resulting in low MCV anemia. Iron is a rate-limiting factor for heme synthesis, a transcriptional regulator of globin synthesis; decreased iron concentration may thus reduce heme synthesis, leading to decreased α-globin precipitation on erythrocyte membranes.[18] In addition, transferrin levels are markedly elevated after thalidomide treatment, and additional transferrin may have the inherent ability to distribute small doses of iron to a large number of RBCs in thalassemia.[18] Patients with TDT may thus benefit from the reductions in MCV and MCH caused by thalidomide treatment.

The mechanism by which thalidomide benefits patients with thalassemia is currently unclear. On the one hand, thalidomide effectively enhanced the expression of *GATA-1* and *EKLF* in erythroid progenitor cells and induced the expression of the γ-globin gene.[20] On the other hand, thalidomide induced γ-globin gene expression and increased HbF synthesis through reactive oxygen species-dependent activation of the p38 mitogen-activated protein kinase signaling pathway and histone H4 acetylation.[7] Thalidomide can also increase the number of hematopoietic colonies, including erythrocyte colonies, and increase demethylation of H3 histone and acetylation of H4 histones in erythroid precursor cells, making it more effective in upregulating HbF.[21,22] In addition, thalidomide promotes erythropoiesis by inducing STAT5 and GATA-1 transcription factors.[23] The current results showed that thalidomide improved erythropoiesis and iron homeostasis to some extent. The effects of thalidomide on thalassemia are thus multifaceted, and more comprehensive research is needed to elucidate the key targets and pathways of thalidomide in treating thalassemia, including a long-term study in a larger cohort of TDT patients.

Conclusions. In summary, our findings demonstrate that thalidomide improves TDT, possibly via improvements in erythropoiesis and iron homeostasis. This study expands our understanding of the effects of thalidomide in thalassemia and provides evidence to support its use in treating this disease.

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