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ORIGINAL ARTICLE

miR-144 regulates oxidative stress tolerance of thalassemic erythroid cell via targeting *NRF2*

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Abstract

Thalassemia has a high prevalence in Thailand. Oxidative damage to erythroid cells is known to be one of the major etiologies in thalassemia pathophysiology. Oxidative stress status of thalassemia is potentiated by the heme, nonheme iron, and free iron resulting from imbalanced globin synthesis. In addition, levels of antioxidant proteins are reduced in α-thalassemia and β-thalassemia erythrocytes. However, the primary molecular mechanism for this phenotype remains unknown. Our study showed a high expression of miR-144 in β- and α-thalassemia. An increased miR-144 expression leads to decreased expression of nuclear factor erythroid 2-related factor 2 (*NRF2*) target, especially in α-thalassemia. In α-thalassemia, miR-144 and *NRF2* target are associated with glutathione level and anemia severity. To study the effect of miR-144 expression, the gain-loss of miR-144 expression was performed by miR inhibitor and mimic transfection in the erythroblastic cell line. This study reveals that miR-144 expression was upregulated, whereas *NRF2* expression and glutathione levels were decreased in comparison with the untreated condition after miR mimic transfection, while the reduction of miR-144 expression contributed to the increased *NRF2* expression and glutathione level compared with the untreated condition after miR inhibitor transfection. Moreover, miR-144 overexpression leads to significantly increased sensitivity to oxidative stress at indicated concentrations of hydrogen peroxide (H_2O_2) and rescued by miR-144 inhibitor. Taken together, our findings suggest that dysregulation of miR-144 may play a role in the reduced ability of erythrocyte to deal with oxidative stress and increased RBC hemolysis susceptibility especially in thalassemia.

Keywords miR-144 · Oxidative stress · Thalassemia · Hemolysis

Introduction

β- and α-thalassemia are the most common autosomal recessive disorders resulting from defects in the biosynthesis of the β- and α-globin chain, respectively. The imbalance ratio of globin chain synthesis leads to excessive β_4- or unmatched α-globin chain which caused red blood cell (RBC) damage, ineffective erythropoiesis, and shortened red cell survival

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[1]. An unstable of globin chain leads to heme, nonheme iron, and free iron formation in RBCs. It results in reactive oxygen species (ROS) generation in thalassemic RBCs such as hydroxyl radical [2–4] through a Fenton reaction [5]. Conversely, the reduction of the antioxidant level of β-thalassemia and α-thalassemia such as glutathione (GSH) and vitamins A, C, and E resulting in increased oxidative stress was observed [6–11].

miRNAs are small noncoding RNAs that usually inhibit protein production by binding the 3′ untranslated region (UTR) of specific target mRNAs via perfect and imperfect binding sequence [12, 13]. The miRNA regulation of oxidative status was demonstrated [14, 15]. Sickle cell disease (SCD) shows an increased level of oxidative stress and lower antioxidant capacity. Previous study reveals association between high erythrocytic miR-144 expression and severity of anemia [16]. According to this study, miR-144 directly modulates oxidative stress through transcription nuclear factor erythroid 2-related factor 2 (NRF2), a basic leucine zipper transcription factor. Association between increased miR-144 and reduced NRF2 levels in homozygous Hb S (HbSS) reticulocytes together with decreased glutathione regeneration was demonstrated [16].

NRF2 is one of the central regulators responding to antioxidant production. Under oxidative stress, NRF2 binds to the antioxidant response element (ARE) that resides in promoters of genes involved in oxidative stress response [17, 18]. The binding of NRF2 to ARE sequence importantly induces antioxidant enzyme expression such as superoxide dismutase (SOD), catalase (CAT), GPX1, phase II detoxification enzymes, NAD(P)H, quinone oxidoreductase (NQO1), and GSH synthesis [19–21]. In addition to antioxidant enhancement, NRF2 also induces the expression of heme oxygenase-1 (HO-1) acting as rate-limiting enzyme of heme degradation [22]. NRF2 plays an important role in the defense mechanism against oxidative stress of RBCs. Targeted deletions of NRF2 cause the development of immune-mediated hemolytic anemia due to increased sensitivity to oxidative stress, decreased GSH levels, and damaged erythrocytes [23]. Double heterozygous knockout, selenocysteine-tRNA gene (*Trsp*) and *Nrf2* gene, dramatically enhances the anemic status and increases intracellular hydrogen peroxide levels in erythroblasts [24].

In SCD erythrocyte, upregulated miR-144 expression leads to decreased *NRF2* expression and glutathione level. Whether miR-144 play a role in the oxidative stress of thalassemia is still unclear.

Data gathered in the current work demonstrated the regulatory effect of miR-144 on *NRF2* expression which is important for oxidative stress tolerance in thalassemic erythrocyte cell.

In this report, the reticulocyte miR-144 expression of β- and α-thalassemia was analyzed. We found a high expression of miR-144 in β- and α-thalassemia. The gain of miR-144 expression results in decreased expression of *NRF2* target gene and GSH levels. Conversely, reduction of miR-144 expression contributed to the increased *NRF2* expression and glutathione level compared with the untreated condition after miR-inhibitor transfection. Taken together, our findings suggest that the regulatory effect of miR-144 on *NRF2* expression is important for oxidative stress tolerance in thalassemic erythrocyte cell.

Methods

Subjects

Twenty-five individual 6-ml EDTA blood samples were obtained (six normal samples, nine HbH diseases (HbH), three HbH-CS disease (HbH-CS), and seven β-thalassemia/HbE) from the Department of Pediatrics, Faculty of Medicine, Prince of Songkla University. Patients received a blood transfusion once every 4 months. All blood samples were collected immediately before each blood transfusion. This study was performed in accordance with the Helsinki Declaration and was approved by the Ethics Committee, Faculty of Medicine, Prince of Songkla University (approval ID 57-217-19-2). Written informed consent was obtained from all individual participants included in the study.

Hematologic study and morphological study of red blood cells

A complete blood count of 25 individual samples was determined using the automated cell counter (Sysmex XN-3000, Kobe, Japan) (Table 1). Blood smears were stained with Wright-Giemsa and analyzed by light microscope.

Reticulocyte collection

EDTA blood was centrifuged at 5000 rpm, 4 °C for 5 min to remove the plasma and buffy coat. The packed red cells were washed twice with a cold saline solution and centrifuged at 12,000 rpm, 4 °C for 20 min two times to collect the reticulocyte portion that predominantly located in upper portion of pack red cells [25]. The reticulocye purity was examined by CD71 transferrin receptor fluorescence marker and detected by flow cytometry and stained with methylene blue staining. The CD 71 positive cell enrichment of post-separation showed 2.1–2.5 times compared that of pre-separation. By methylene blue staining, the percent of reticulocyte post-separation result was 1.5–2.3 times compared that of pre-separation.

Cell culture

K562 cells, a chronic myelogenous leukemia-derived cell line, were maintained in RPMI 1640 medium with 10% heat-inactivated fetal bovine serum (FBS, GIBCO, Invitrogen, NY, USA), 2 mM glutamine, and antibiotics under 5% CO_2, 37 °C condition [16].

Table 1 RBC properties of normal control, Hb H disease, HbH CS disease, and β-thalassemia/HbE

Red blood parameter	Normal ($n = 6$)	Hb H disease ($n = 9$)	HbH CS disease ($n = 3$)	β-Thalassemia/HbE ($n = 7$)
RBC count ($\times 10^6$ cell/L)	5.1 ± 0.4	4.5 ± 0.8	3.8 ± 0.1**	3.3 ± 0.6**
Hb (g/dL)	13.9 ± 1.1	8.5 ± 1.2**	7.8 ± 0.2**	7.9 ± 1.4**
Hct (%)	42.5 ± 3.3	29.8 ± 4.3**	29.6 ± 2.0**	24.1 ± 4.4 **
MCV (fL)	84.0 ± 2.1	67.3 ± 9.2**	78.8 ± 2.7*	72.8 ± 4.5 **
MCH (pg)	27.4 ± 1.0	19.1 ± 2.3**	20.9 ± 1.3**	23.9 ± 2.0**
MCHC (g/dL)	32.6 ± 0.4	28.54 ± 1.5**	26.6 ± 2.4**	32.8 ± 0.9
RDW (%)	13.6 ± 0.7	24.5 ± 2.3**	24.1 ± 3.9**	24.7 ± 4.7 **
Reticulocyte (%)	1.6 ± 0.6	5.0 ± 2.4**	8.8 ± 1.6**	5.2 ± 3.2*
ROS level (MFI)	30.8 ± 5.9	96.7 ± 42.9**	78.7 ± 30.3**	80.4 ± 12.5**
GSH (MFI)	430 ± 146.5	199.1 ± 95.3**	148.3 ± 46.6*	194.0 ± 57.1**

Data expressed as mean ± SD. The significant data were shown in values with *$p < 0.05$ and **$p < 0.01$ using independent Student's t test when compared with normal individuals

RBC red blood cells, *Hb* hemoglobin, *Hct* hematocrit, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *RDW* red cell distribution width, *ROS* reactive oxygen species, *GSH* glutathione

ROS and GSH assays

To determine oxidative status, ROS assay and GSH assay were measured in erythrocyte (52). ROS levels were quantified by dichlorofluorescein (DCF) assay. 2′,7′-Dichlorofluorescein diacetate (DCFH-DA) is a cell membrane permeable substance. A DCFH-DA is deacetylated by intracellular esterase. DCFH is changed to a fluorescent product, 2′,7′ dichlorofluorescein (DCF), through the oxidation of H_2O_2 following the method described previously [26]. The blood sample (1×10^6 cell/ml) or K562 cells (2×10^5 cell) were incubated with DCFH-DA (Sigma-Aldrich, Germany) dissolved in methanol at a final concentration of 0.4 mM. After incubation at 37 °C for 15 min in a humidified atmosphere of 5% CO_2, the cells were washed and re-suspended in PBS (GIBCO, Invitrogen). Then, the samples were stimulated with 2 mM of freshly prepared H_2O_2 at room temperature for 30 min. The ROS levels were analyzed by flow cytometry (BD FACSCalibur™, BD Bioscience) and calculated as mean fluorescence intensity (MFI) using CellQuest software.

For GSH assay, GSH was measured using mercury orange (Sigma-Aldrich) stain modified from a previous protocol [26, 27]. The blood sample (2×10^6 cell/ml) or K562 cells (3×10^5 cell) were washed with PBS and spun down. Then, the pellet was incubated with freshly prepared of 2 mM H_2O_2 at 37 °C for 1 h in a humidified atmosphere of 5% CO_2. Then, the cell pellet was incubated for 3 min with 40 μM of mercury orange that was prepared from 100 μM stock solution dissolved by acetone. Finally, the samples were washed and re-suspended in PBS. GSH levels were analyzed by flow cytometry (BD FACSCalibur™, BD Bioscience) and determined as mean fluorescence intensity (MFI) using CellQuest software.

miRNA and target gene expression

Total RNAs were extracted from partially reticulocyte enriched and K562 cell line using the Hybrid-R™ miRNA Isolation Kit (GeneAll, Seoul, South Korea) according to the manufacturer's instructions. The concentration of RNA was measured with a NanoDrop 2000 spectrophotometer (Thermo Scientific, DE, USA). Mature miRNA expression specific for miR-144 was evaluated by TaqMan® Small RNA Assays (Applied Biosystems, Foster City, CA, USA) and using RNU48 as a reference gene. Briefly, total RNA was reversely transcribed using the specific looped primer; then, reverse transcription quantitative PCR was conducted using the Taqman miRNA assay protocol. Amplification of miRNA by qRT-PCR was detected by LightCycler® 480 PCR System (Roche Molecular System, CA, USA). The experiments were carried out in triplicate. The expression of miRNA normalized on reference gene was calculated using $2^{-\Delta\Delta Ct}$ (comparative Ct) method by using normal or untreated as control.

To determine the target of miR-144, NRF2 [16], the mRNA target was analyzed by qRT-PCR. Total RNAs extracted from partially reticulocyte enriched and K562 cell line were subjected to reverse transcription to complementary DNA (cDNA) using a High-Capacity RNA-to-cDNA Kit (Applied Biosystems) according to established protocols. The amplification of cDNAs by the qRT-PCR method was done using SYBR Premix EX Taq™ (Takara Bio, Shiga, Japan) with specific primers, *NRF2*: forward primer, 5′-GACG GTATGCAACAGGACATTGAG-3′, reverse primer, 5′-AACTTCTGTCAGTTTGGCTTCTGGA-3′ and *GAPDH*: forward primer, 5′-GAAGGTGAAGGTCGGAGTC-3′, reverse primer, 5′-GAAGATGGTGATGGGAT TTC-3′.

Amplification was performed with an initial denaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 3 s, annealing at 60 °C for 30 s, and melt curve stage. GAPDH served as the internal control. Normalized values ($2^{-\Delta\Delta Ct}$) were compared among samples, and the experiments were carried out in triplicate.

miR-144 mimic and anti-miR-144 inhibitor transfection

To study the effects of miR-144, gain and loss were performed. K562 cells/well (2×10^5) with 40 nM hsa-miR-144 miRNA mimic and negative mimic control (mirVana™ miRNA mimic; Applied Biosystem) were used to increase miRNA activity. miR-144 inhibitor with 400 nM (anti-miR™ miRNA inhibitor; Applied Biosystem) was used for inhibition of miRNA activity. miRNA mimic and inhibitor that were transfected into K562 cells were done using RNAiMAX (Invitrogen). Cells were harvested at 72 h after transfection. The expression of miR-144 and its target were determined by qRT-PCR. The levels of ROS and GSH assays were measured by flow cytometry.

Statistical analysis

Data was presented as means plus standard deviation (SD). Student's *t* test was used to calculate statistical significance. A *p* value less than 0.05 was considered statistically significant.

Results

Upregulation of miR-144 and downregulation of *NRF2* mRNA target in thalassemic reticulocytes

To study miRNA expression, thalassemic reticulocyte cells were isolated and determined miRNA expression by qRT-PCR. The expression level of miR-144 in reticulocyte cells obtained from HbH disease, HbH-CS disease, and β-thalassemia/HbE individuals was significantly increased—16.4-fold, 31.6-fold, and 3.1-fold, respectively ($p < 0.01$)—when compared with that of normal samples (Fig. 1a). To identify miR-144 regulation, we hypothesized that NRF2 could be functionally appropriate to be the target of miR-144 because of its importance in regulating oxidative stress tolerance and mediating antioxidant production in SCD [16]. The levels of *NRF2* gene expression were determined in reticulocyte derived from HbH disease, HbH-CS disease, and β-thalassemia/HbE individuals. The expression of *NRF2* of HbH disease ($p < 0.01$), HbH-CS disease, and β-thalassemia/HbE ($p < 0.05$) was decreased, compared with that of normal reticulocyte (Fig. 1b).

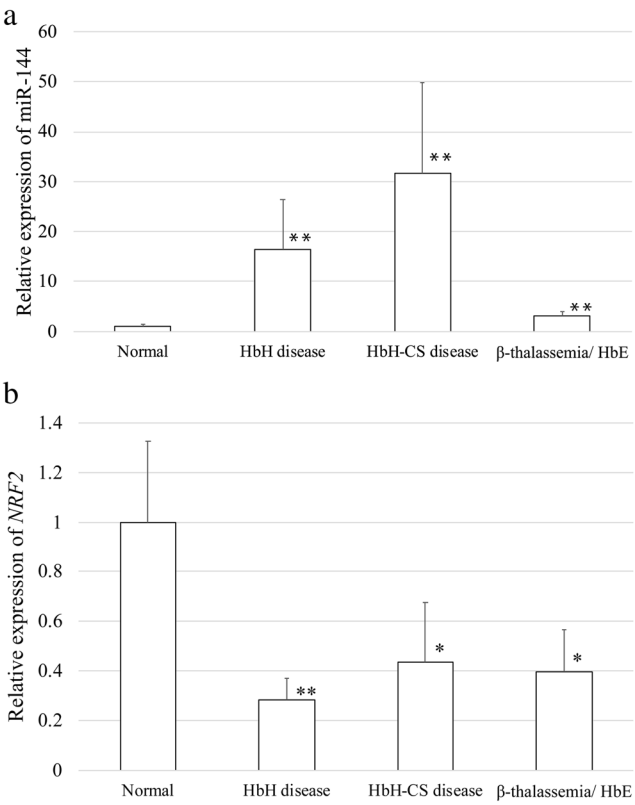

Fig. 1 Analysis of miR-144 and NRF2 mRNA target expression in thalassemic reticulocyte. Expression of miR-144 (**a**) and *NRF2* mRNA target (**b**) in normal ($N = 6$), HbH ($N = 9$), HbH-CS ($N = 3$), and β-thalassemia/HbE ($N = 7$) was carried out by qRT-PCR. *$p < 0.05$; **$p < 0.01$

Overexpression of miR-144 increased erythroid cell response to oxidative stress

To test the functional effect of miR-144 for regulating *NRF2* mRNA target to modulate oxidative stress tolerance, the gain of function of miR-144 was investigated. We transfected miR-144 mimic into K562 cells. The expression of miR-144 was increased compared with that of the untreated and negative control ($p < 0.01$) (Fig. 2a). Our data found that miR-144 overexpression leads to a detectable reduction of *NRF2* mRNA expression ($p < 0.05$) (Fig. 2b).

To further confirm the effect of miR-144 overexpression on oxidative stress status, we measured the ROS and GSH levels by fluorescence intensity. The result showed that ROS levels were increased compared with those of the untreated and negative control ($p < 0.05$) (Fig. 2c). While GSH levels were decreased in miR-144 mimic conditions ($p < 0.05$) (Fig. 2d).

Furthermore, we transfected miR-144 mimic and detected their cell viability under the oxidative stress of different hydrogen peroxide (H_2O_2) concentrations (0, 10, 20, and 30 μM). We found that miR-144 overexpression leads to a decrease in cell viability in 20 and 30 μM H_2O_2-induced oxidative stress ($p = 0.07$ and $p < 0.05$, respectively) when

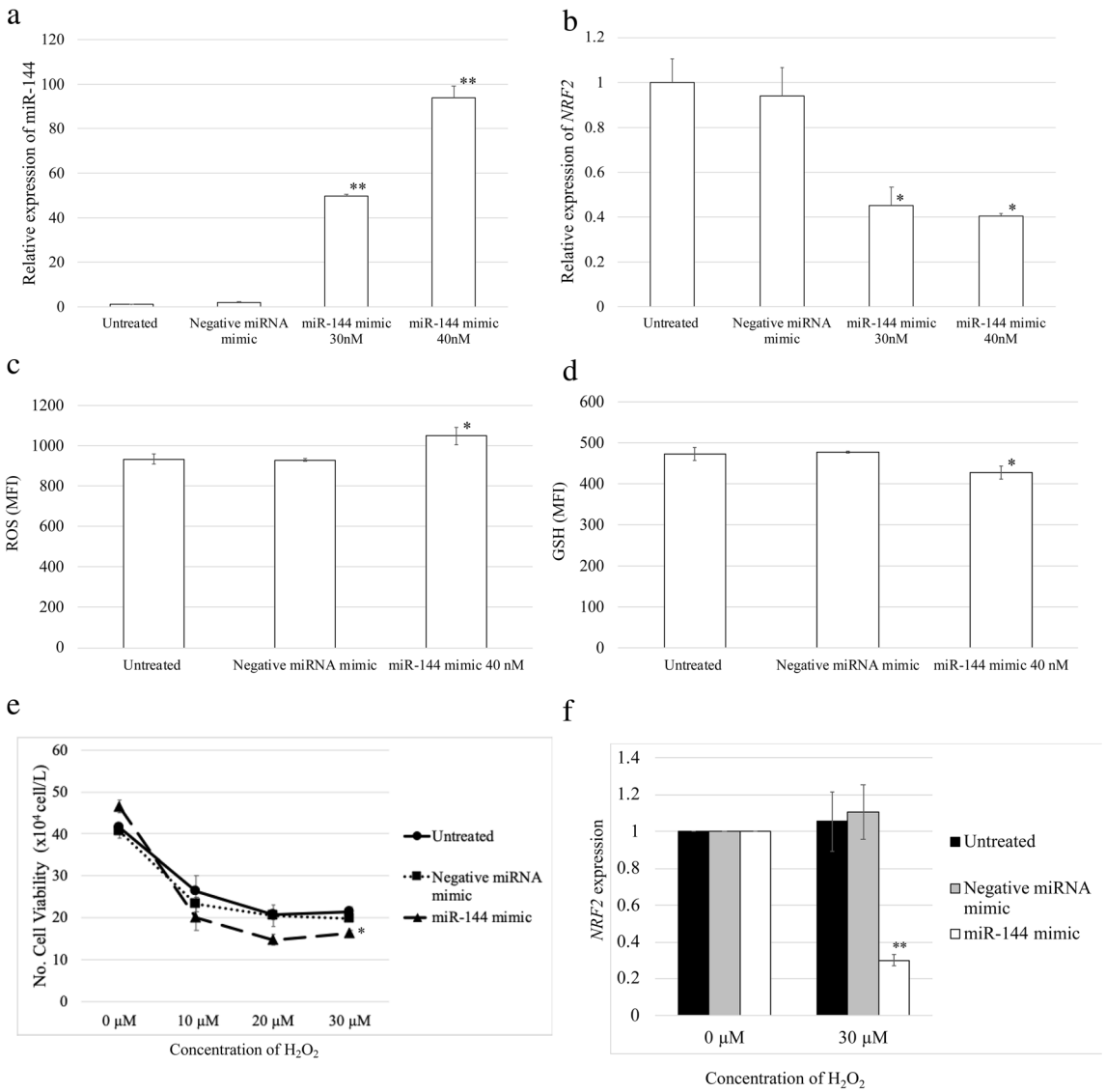

Fig. 2 Gain of miR-144 expression in erythroblastic cell line. Erythroblastic cell line was treated with hsa-miR-144 miRNA mimic and negative control. Expression of miR-144 (**a**) and *NRF2* target gene expression (**b**) was examined by qRT-PCR. The ROS levels (**c**) and glutathione levels (**d**) were demonstrated by flow cytometry. Cell viability as measured by trypan blue staining method (**e**) and expression of *NRF2* target gene (**f**) of K562 erythroblastic cell line in H_2O_2-induced oxidative stress were exhibited in miR-144 mimic treated. Data are means of triplicate determinations. *$p < 0.05$; **$p < 0.01$

compared with that of the untreated sample and negative miRNA mimic (Fig. 2e). The *NRF2* mRNA expression was significantly downregulated in miR-144 overexpression under oxidative stress ($p < 0.01$) (Fig. 2f). These results indicate that miR-144 overexpression-mediated NRF2 repression can play a direct and important role in the reduction of antioxidation in erythroid cells.

The inhibition of miR-144 expression promotes oxidative stress tolerance

To investigate the effect of decreased miR-144 expression, a loss-of-function study was performed using the anti-miR-144. The result showed the transfection of K562 cells. With 300 nM anti-miR-144 caused a significant reduction ($p < 0.01$) (Fig. 3a). At 72 h post-transfection, addition of anti-miR-144 promoted *NRF2* mRNA expression to a level comparable with that obtained from untreated and negative control ($p < 0.01$) (Fig. 3b).

We also studied the effect of miR-144 inhibition on oxidative stress status. Downregulation of miR-144 caused no significant effects to ROS levels (Fig. 3c). However, a significant increase of GSH levels was observed in K562 cells transfected with anti-miR-144 as compared with cells transfected with the untreated and negative control ($p < 0.01$) (Fig. 3d). In addition, we also conducted a loss-of-function study on miR-144 and detected their viability under oxidative stress induced by different hydrogen peroxide (H_2O_2) concentrations. We found

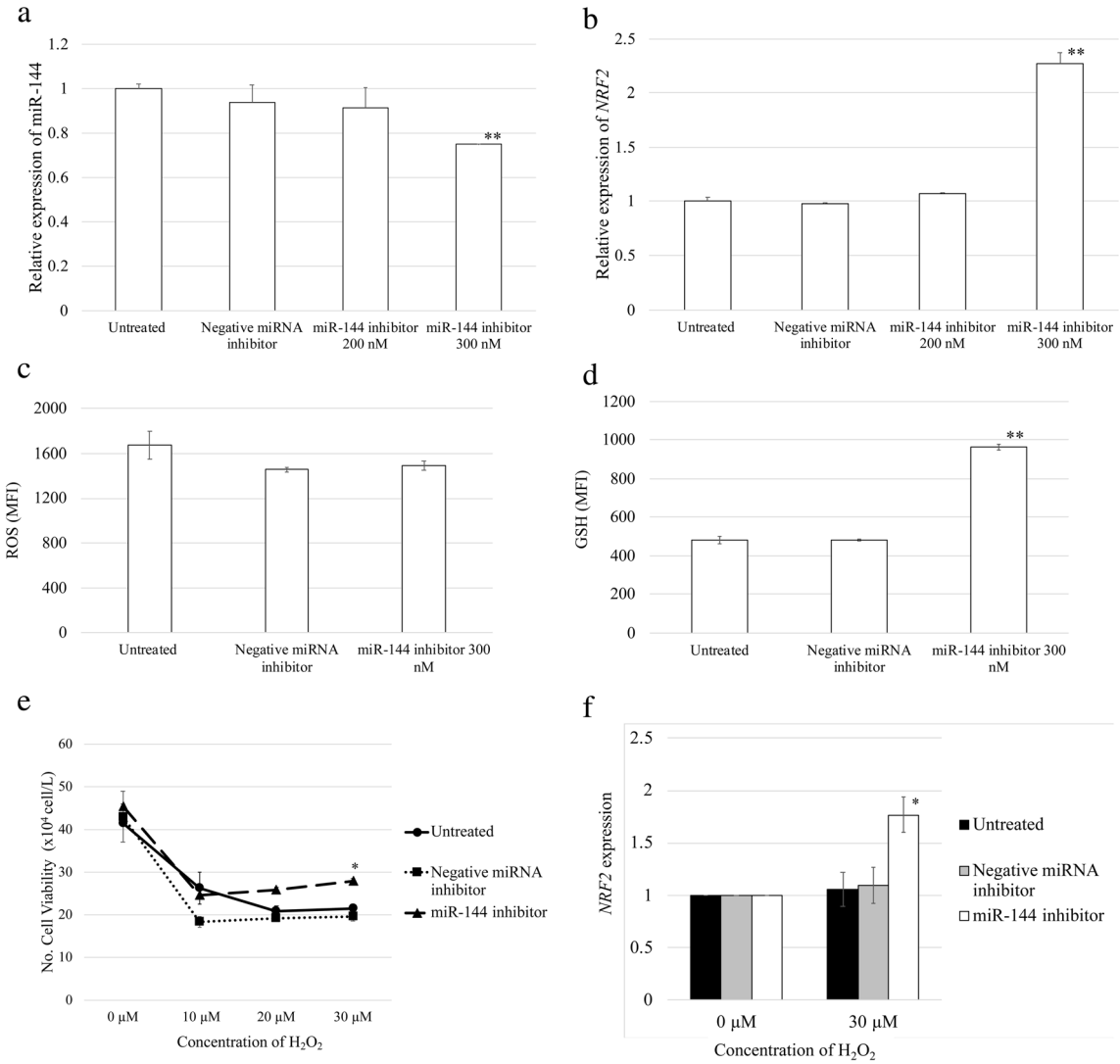

Fig. 3 Loss of miR-144 expression in erythroblastic cell line. Erythroblastic cell line was treated with hsa-miR-144 miRNA inhibitor and negative control. Expression of miR-144 (**a**) and *NRF2* target gene expression (**b**) was examined by qRT-PCR. The ROS levels (**c**) and glutathione levels were demonstrated by flow cytometry. Cell viability as measured by trypan blue staining method (**e**) and expression of *NRF2* target gene (**f**) of K562 erythroblastic cell line in H_2O_2-induced oxidative stress were exhibited in miR-144 inhibitor treated. Data are means of triplicate determinations. *$p < 0.05$; **$p < 0.01$

that the reduction of miR-144 expression leads to significantly increased cell viability in 30 μM H_2O_2-induced oxidative stress ($p < 0.05$) when compared with that of the untreated control and negative control (Fig. 3e). Likewise, the *NRF2* mRNA expression was significantly upregulated in inhibition of miR-144 under oxidative stress ($p < 0.01$) (Fig. 3f). Taken together, these results indicate that NRF2 is a valid target gene of miR-144 to regulate GSH production, especially under oxidative stress.

Discussion

β- and α-thalassemia are inherited disorders with significant phenotypic diversity. The regulation of miRNA in erythrocytes may be important in gaining more information about such different phenotypes and their clinical manifestations. Elevated miR-144 level was detected in mature RBC of SCD patients [16]. Here, we have demonstrated the association of high miR-144 expression in thalassemia reticulocyte, particularly increased expression with a hemolysis phenotype in α-thalassemia.

miRNA microarray analysis of sickle cell anemia red blood cells showed a degree of similarity in the miR-144 level in earlier stages of erythroid differentiation and mature red blood cells [28]. The results from RBC proteomic and transcriptome demonstrated that antioxidant activity of proteins and transcriptional levels mostly remain at the same level during maturation of adult reticulocytes to RBCs [29, 30]. Moreover, previous study demonstrated microassay-based miRNA

expression profile about gene association with HbF expression in SCD patient from reticulocyte [31].

In addition, elevated miR-144 leads to lower expression of Nrf2, resulting in lower expression of genes encoding antioxidant defense proteins. The increased expression of miR-144 leads to a decreased expression level of the NRF2 target gene. This observation is consistent with the hemolytic anemia phenotype seen in SCD patients [16]; targeted deletions of NRF2 mice developed immune-mediated hemolytic anemia [23] and double heterozygous knockout: selenocysteine-tRNA gene (*Trsp*) and *Nrf2* gene [24]. Besides erythroid cells, miR-144 regulated NRF2, and oxidative stress tolerance was observed in human SH-SY5Y cells as a neuroblastic subclone of the neuroblastoma cell line SK-N-SH [32].

We provided evidence of the significance of the dysregulated miR-144-NRF2 regulatory axis in the oxidative stress tolerance among thalassemia patients with α-thalassemia hemolysis phenotype. Erythroid cells with high miR-144 expression exhibited lower NRF2 levels, decreased GSH, and higher susceptibility to oxidative damage, which is similar to the SCD study [16]. The gain of miR-144 expression resulted in decreased expression of *NRF2* target gene and GSH levels. The augmented sensitivity to oxidative stress in increased miR-144 was rescued by miR-144 inhibitor. Our findings suggest that the regulatory effect of miR-144 on *NRF2* expression is important for oxidative stress tolerance in thalassemic erythrocyte cell.

Clinical implications Our observations illustrated that erythroid miRNAs and its target may contribute to the complex regulatory network of disease modifiers in thalassemia pathophysiology and can support in the association and observation of disease heterogeneity and anemia severity. It is likely to benefit from antioxidant treatments. In addition to thalassemia, other anemia disorders (e.g., glucose-6-phosphate dehydrogenase deficiency or hereditary spherocytosis) also manifest enhanced oxidative stress and impaired antioxidant status in their erythrocytes. It will be important to determine whether the dysregulation of miR-144 may also play a role in their reduced ability to deal with oxidative stress and susceptibility to hemolysis in those disorders.

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Authorship KS was the principal investigator and takes primary responsibility for contributed to apply for funding, the study design, performed the experiment, interpretation of the data, drafting, and editing of the manuscript. NS contributed to the study design and the editing of the manuscript. KP and MW were responsible for specimen collection and the editing of the manuscript. SS contributed to the perform experiment and the editing of the manuscript. SF contributed to the study design and the editing of the manuscript. The final version of the article to be published was read and approved by all authors.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Redundant publication No substantial overlap with previous papers.

References

1. Yuan J, Bunyaratvej A, Fucharoen S, Fung C, Shinar E, Schrier SL (1995) The instability of the membrane skeleton in thalassemic red blood cells. Blood 86(10):3945–3950
2. Advani R, Rubin E, Mohandas N, Schrier SL (1992) Oxidative red blood cell membrane injury in the pathophysiology of severe mouse beta-thalassemia. Blood 79(4):1064–1067
3. Advani R, Sorenson S, Shinar E, Lande W, Rachmilewitz E, Schrier SL (1992) Characterization and comparison of the red blood cell membrane damage in severe human alpha- and beta-thalassemia. Blood 79(4):1058–1063
4. Schrier SL (2002) Pathophysiology of thalassemia. Curr Opin Hematol 9(2):123–126
5. Sadrzadeh SM, Graf E, Panter SS, Hallaway PE, Eaton JW (1984) Hemoglobin. A biologic Fenton reagent. J Biol Chem 259(23): 14354–14356
6. Kalpravidh RW, Tangjaidee T, Hatairaktham S, Charoensakdi R, Panichkul N, Siritanaratkul N, Fucharoen S (2013) Glutathione redox system in beta-thalassemia/Hb E patients. Sci World J 2013:543973. https://doi.org/10.1155/2013/543973
7. Kassab-Chekir A, Laradi S, Ferchichi S, Haj Khelil A, Feki M, Amri F, Selmi H, Bejaoui M, Miled A (2003) Oxidant, antioxidant status and metabolic data in patients with beta-thalassemia. Clin Chim Acta 338(1–2):79–86
8. Meral A, Tuncel P, Surmen-Gur E, Ozbek R, Ozturk E, Gunay U (2000) Lipid peroxidation and antioxidant status in beta-thalassemia. Pediatr Hematol Oncol 17(8):687–693
9. Cheng ML, Ho HY, Tseng HC, Lee CH, Shih LY, Chiu DT (2005) Antioxidant deficit and enhanced susceptibility to oxidative damage in individuals with different forms of alpha-thalassaemia. Br J Haematol 128(1):119–127
10. Chiou SS, Tsao CJ, Tsai SM, Wu YR, Liao YM, Lin PC, Tsai LY (2014) Metabolic pathways related to oxidative stress in patients with hemoglobin h disease and iron overload. J Clin Lab Anal 28(4):261–268
11. Mirlohi MS, Yaghooti H, Shirali S, Aminasnafi A, Olapour S (2018) Increased levels of advanced glycation end products positively correlate with iron overload and oxidative stress markers in patients with beta-thalassemia major. Ann Hematol 97(4):679–684. https://doi.org/10.1007/s00277-017-3223-3
12. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116(2):281–297
13. Carthew RW, Sontheimer EJ (2009) Origins and mechanisms of miRNAs and siRNAs. Cell 136(4):642–655
14. Siow RC, J Forman H (2013) Redox regulation of microRNAs in health and disease. Free Radic Biol Med 64:1–3
15. Nallamshetty S, Chan SY, Loscalzo J (2013) Hypoxia: a master regulator of microRNA biogenesis and activity. Free Radic Biol Med 64:20–30

16. Sangokoya C, Telen MJ, Chi JT (2010) microRNA miR-144 modulates oxidative stress tolerance and associates with anemia severity in sickle cell disease. Blood 116(20):4338–4348
17. Ishii T, Itoh K, Takahashi S, Sato H, Yanagawa T, Katoh Y, Bannai S, Yamamoto M (2000) Transcription factor Nrf2 coordinately regulates a group of oxidative stress-inducible genes in macrophages. J Biol Chem 275(21):16023–16029
18. Motohashi H, Yamamoto M (2004) Nrf2-Keap1 defines a physiologically important stress response mechanism. Trends Mol Med 10(11):549–557
19. Harvey CJ, Thimmulappa RK, Singh A, Blake DJ, Ling G, Wakabayashi N, Fujii J, Myers A, Biswal S (2009) Nrf2-regulated glutathione recycling independent of biosynthesis is critical for cell survival during oxidative stress. Free Radic Biol Med 46(4):443–453
20. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, Nabeshima Y (1997) An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun 236(2):313–322
21. Chan JY, Kwong M (2000) Impaired expression of glutathione synthetic enzyme genes in mice with targeted deletion of the Nrf2 basic-leucine zipper protein. Biochim Biophys Acta 1517(1):19–26
22. Kitamuro T, Takahashi K, Ogawa K, Udono-Fujimori R, Takeda K, Furuyama K, Nakayama M, Sun J, Fujita H, Hida W, Hattori T, Shirato K, Igarashi K, Shibahara S (2003) Bach1 functions as a hypoxia-inducible repressor for the heme oxygenase-1 gene in human cells. J Biol Chem 278(11):9125–9133
23. Lee JM, Chan K, Kan YW, Johnson JA (2004) Targeted disruption of Nrf2 causes regenerative immune-mediated hemolytic anemia. Proc Natl Acad Sci U S A 101(26):9751–9756
24. Kawatani Y, Suzuki T, Shimizu R, Kelly VP, Yamamoto M (2011) Nrf2 and selenoproteins are essential for maintaining oxidative homeostasis in erythrocytes and protecting against hemolytic anemia. Blood 117(3):986–996
25. Srinoun K, Svasti S, Chumworathayee W, Vadolas J, Vattanaviboon P, Fucharoen S, Winichagoon P (2009) Imbalanced globin chain synthesis determines erythroid cell pathology in thalassemic mice. Haematologica 94(9):1211–1219
26. Amer J, Goldfarb A, Fibach E (2004) Flow cytometric analysis of the oxidative status of normal and thalassemic red blood cells. Cytometry A 60(1):73–80
27. Amer J, Ghoti H, Rachmilewitz E, Koren A, Levin C, Fibach E (2006) Red blood cells, platelets and polymorphonuclear neutrophils of patients with sickle cell disease exhibit oxidative stress that can be ameliorated by antioxidants. Br J Haematol 132(1):108–113
28. Chen SY, Wang Y, Telen MJ, Chi JT (2008) The genomic analysis of erythrocyte microRNA expression in sickle cell diseases. PLoS One 3(6):e2360
29. Goh SH, Josleyn M, Lee YT, Danner RL, Gherman RB, Cam MC, Miller JL (2007) The human reticulocyte transcriptome. Physiol Genomics 30:172–178
30. Wilson MC, Trakarnsanga K, Heesom KJ, Cogan N, Green C, Toye AM, Parsons SF, Anstee DJ, Frayne J (2016) Comparison of the proteome of adult and cord erythroid cells, and changes in the proteome following reticulocyte maturation. Mol Cell Proteomics 15(6):1938–1946
31. Li B, Zhu X, Ward CM, Starlard-Davenport A, Takezaki M, Berry A, Ward A, Wilder C, Neunert C, Kutlar A, Pace BS (2019) MIR-144-mediated NRF2 gene silencing inhibits fetal hemoglobin expression in sickle cell disease. Exp Hematol 70:85–96
32. Zhou C, Zhao L, Zhenga J (2017) MicroRNA-144 modulates oxidative stress tolerance in SH-SY5Y cells by regulating nuclear factor erythroid 2-related factor 2-glutathione axis. Neurosci Lett 655: 21–27

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