ORIGINAL

A high concentration of triiodothyronine attenuates the stimulatory effect on hemin-induced erythroid differentiation of human erythroleukemia K562 cells

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Abstract. Although thyroid hormone is a known stimulator of erythropoietic differentiation, severe anemia is sometimes observed in patients with hyperthyroidism and this mechanism is not fully understood. The aim of this study was to investigate the effect of triiodothyronine (T3) on hemin-induced erythropoiesis. Human erythroleukemia K562 cells were used as an erythroid differentiation model. Cell differentiation was induced by hemin and the effect of pre-incubation with T3 (0.1 to 100 nM) was analyzed by measuring the benzidine-positive rate, hemoglobin content, CD71 expression (transferrin receptor), and mRNA expression for transcription factors related to erythropoiesis and thyroid hormone receptors (TRs). Hemin, a promoter of erythroid differentiation, increased the levels of mRNAs for TR α , TR β , and retinoid X receptor α (RXR α), as well as those for nuclear factor-erythroid 2 (NFE2), GATA-binding protein 1 (GATA1) and GATA-binding protein 2 (GATA2). Lower concentrations of T3 had a stimulatory effect on hemin-induced hemoglobin production (1 and 10 nM), CD71 expression (0.1 nM), and α -globin mRNA expression (1 nM), while a higher concentration of T3 (100 nM) abrogated the stimulatory effect on these parameters. T3 at 100 nM did not affect cell viability and proliferation, suggesting that the abrogation of erythropoiesis enhancement was not due to toxicity. T3 at 100 nM also significantly inhibited expression of GATA2 and RXR α mRNA, compared to 1 nM T3. We conclude that a high concentration of T3 attenuates the classical stimulatory effect on erythropoiesis exerted by a low concentration of T3 in hemin-induced K562 cells.

Key words: Triiodothyronine (T3), K562 cells, Erythroid differentiation, Hemin, Thyroid hormone(s)

THYROID hormone stimulates erythropoiesis and clinical hypothyroidism is often complicated by reductions in red cell mass and total blood volume, and by normocytic normochromic anemia [1–4]. Clinically, bone marrow has been observed to show erythroid hyperplasia in patients with hyperthyroidism [5]. However, anemia is sometimes observed in patients with hyperthyroidism such as Graves' disease (GD). Some reports indicate that anemia associated with hyperthyroidism occurs in 10–22% of cases of non-treated hyperthyroidism, and that these cases recover well with treatment of

hyperthyroidism [6, 7]. This type of anemia is found most often in patients with hyperthyroidism of unusual severity or prolonged duration [8].

Thyroid hormone has a direct positive action on bone marrow erythropoiesis in rats [9] and thyroid hormone receptor α (TR α) was found to be essential for regulating erythropoiesis in a study characterizing the phenotype of TR α ^{-/-} mice [10]. Bauer *et al.* showed that thyroid hormone receptor functions as a developmental switch in erythroid differentiation and that T3 suffices to arrest proliferation and induce erythropoiesis [11]. However, the mechanisms underlying the association between anemia and hyperthyroidism are not completely clear.

The K562 cell line was established from the pleural effusion of a 53-year-old woman as the first human

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immortalized leukemia cell line [12]. K562 cells are widely used as a model for investigation of erythropoiesis and globin gene expression. Differentiation of these cells can be induced by chemical agents such as hemin, sodium butyrate, DMSO, and erythropoietin (Epo) [13–15]. Hemin is an iron-containing porphyrin that is the prosthetic moiety for a broad range of proteins involved in oxygen delivery and is used to treat acute porphyria and thalassemia intermedia [16]. Hemin is a relatively strong inducer of heme biosynthesis among various chemical inducers of erythroid differentiation of K562 cells [17, 18].

Recently, we found that an excess of T3 suppresses activin-induced differentiation in erythroleukemia K562 cells under hypoxia, and that GATA2 expression might be correlated with this effect [19]. In this study, we investigated whether T3 has an effect on hemin-induced erythropoiesis in K562 cells, with the goal of elucidating the mechanism underlying anemia in hyperthyroidism.

Materials and Methods

Most of the following procedures have also been described elsewhere [19].

Cell culture

K562 cells were kindly provided by Prof. Kimura (Department of Hematology, National Defense Medical College, Japan). These cells were cultured in RPMI 1640 medium (Life Technologies, Carlsbad, CA) supplemented with 10% (v/v) fetal bovine serum at 37°C in humidified air with 5% CO₂. Hemin (Sigma, St. Louis, MO) was prepared as a 5 mM stock solution in 0.1 N NaOH and stored in aliquots at -20°C. Hemin (40 µM) was used for differentiation unless otherwise noted. Triiodothyronine (T3; Sigma) was prepared as a 1×10⁻⁵ M stock solution in 0.1 N NaOH and stored in aliquots at -20°C. Dilutions were made in RPMI 1640 medium. Thompson *et al.* showed that the transcriptional activities of thyroid hormone receptors α and β are dose dependent from 1 to 100 nM T3 [20], while the threshold T3 concentration for non-genomic effects ranges from 0.1 to 30 nM [21]. Therefore, 0.1 to 100 nM T3 was used for pre-incubation in most experiments in the study.

Growth potential

Cells were harvested at 5×10⁴ cells/mL in a 100 mm

dish and 1 nM and 100 nM T3 or an equal amount of 0.1N NaOH as a negative control were added. At 48 h and 96 h after treatment, 10 µL of medium containing cells was collected and mixed with 10 µL of 0.4% trypan blue solution, after which the number of viable cells was determined using an automated cell counter (TC-20, Bio-rad). At 48 h after determining the cell number, cells were resuspended in the medium and then replated at 5×10⁴ cells/mL. To examine the influence of hemin, K562 cells were harvested at 2×10⁵ cells/mL in a 100 mm dish and 40 µM hemin or an equal amount of 0.1N NaOH was added in the cell culture. At 72 h after treatment, cell viability was determined using an automated cell counter based on trypan blue exclusion.

Flow cytometry

K562 cells were harvested and resuspended at 1×10⁷ cells/mL in Dulbecco's phosphate buffered saline (DPBS). Erythroid cell differentiation was determined by expression of transferrin receptor (CD71) and glycophorin A (CD235a). Cells were incubated with murine-specific monoclonal antibodies directed against CD71 and CD235a. Antibodies were coupled to R-phycoerythrin or fluorescein isothiocyanate. All antibodies were purchased from eBioscience (San Diego, CA). The fluorescence intensity was analyzed using a Cytomics FC 500 instrument (Beckman Coulter, Brea, CA). Results were also recorded as mean fluorescence intensity (MFI), which represents the cell surface receptor density.

Benzidine staining

Benzidine staining was performed as previously described [22]. Briefly, cells (2×10⁴) were seeded into each well of a 6-well dish containing 2 mL of medium. After several conditioning steps, cells were harvested in 10 µL DPBS. Benzidine dihydrochloride solution (2 mg/mL in 0.5% acetic acid) containing 0.15% hydrogen peroxide was added directly to an equal volume (10 µL) of the cell suspension and incubated at 37°C for 5 min. Positively stained cells were counted under a microscope. Images were recorded with an Axio Imager A2 microscope (Carl Zeiss Microscopy, Oberkochen, Germany) equipped with an AxioCam cooled-charge-coupled device camera system operated with AxioVision software (Carl Zeiss Microscopy).

Hemoglobin content

Cells in each dish were collected and cell numbers

were determined using an automated cell counter with trypan blue exclusion (TC-20, Bio-rad). After washing with DPBS twice, 5×10^6 cells were dissolved in 120 μ L of deionized distilled water. Hemoglobin content was measured using a QuantiChrom Hemoglobin Assay Kit (BioAssay Systems, Hayward, CA).

RT-PCR and quantitative real-time RT-PCR

For real-time RT-PCR, total RNA was isolated with Trizol[®] reagent (Life Technologies). cDNA was synthesized from 1 μ g total RNA using SuperScript VILO[®] (Life Technologies). mRNA levels of various genes were analyzed by qRT-PCR using TaqMan[®] Gene Expression Assays (Applied Biosystems, Foster City, CA) with a 7500 Real-Time PCR System (Applied Biosystems). The relative RNA level of each target gene was normalized to the RNA level of peptidylprolyl isomerase A (PPIA; cyclophilin A) as an internal control.

Statistical analysis

Data with a normal distribution are expressed as means $\pm$ SD. Other data are expressed as the median (1st-3rd quartile). Statistical analysis was performed using JMP 10.0.0 (SAS Institute Inc.). Comparison between two groups was performed by Mann-Whitney U test. Three groups were compared using standard one-way ANOVA after establishment of normality in a Shapiro-Wilk test and equal dispersion by Bartlett test. $p < 0.05$ was considered to be significant. A Tukey-Kramer test with Bonferroni correction was performed as a *post hoc* test. Data that were not normally distributed were evaluated using non-parametric analysis (median test or Kruskal Wallis test) followed by a *post hoc* test (Steel-Dwass test or Kruskal-Wallis test) with Bonferroni correction.

Results

Characteristics of K562 cells

The expression levels of mRNAs for TR α , TR β , and retinoid X receptor α (RXR α) in steady state K562 cells were analyzed using quantitative real-time RT-PCR (Fig. 1), based on our previous identification of these mRNAs in the cells [19]. Expression levels were normalized based on the level of cyclophilin A (PPIA) as an internal control. The expression level of RXR α , which forms a heterodimer with TR and significantly enhances binding to thyroid hormone response elements (TREs), was higher than those of TR α and

Fig. 1 Quantification of mRNA expression in steady state (untreated) K562 cells by quantitative real-time PCR for thyroid hormone receptor α (TR α), thyroid hormone receptor beta (TR β), and retinoid X receptor (RXR α). PPIA was used as an internal control. All mRNAs were expressed in K562 cells.

TR β in these cells.

Potential of K562 cells as erythroid progenitors was evaluated through addition of hemin in 100-mm dishes, with an equal amount of 0.1 N NaOH (the vehicle) used as a negative control. After exposure to hemin for 72 h, the benzidine-positive rate was significantly higher in hemin-treated K562 cells compared to non-treated cells (71.0% (70.25-74%) vs. 1.1% (0.9-1.3%), $p=0.032$; hemin: $n=4$, control: $n=3$), consistent with previous studies [23, 24]. Hemoglobin levels per 5×10^6 cells after exposure to 10 to 50 μ M hemin for 72 h were elevated in a dose-dependent manner in K562 cells, with a significant difference at 40 and 50 μ M compared to control cells (Fig. 2A).

To analyze the influence of hemin on the cell proliferation rate and viability, cell counts were determined after hemin treatment. The cell count was significantly lower after exposure to 40 μ M hemin for 72 h compared to controls ($6.72 \pm 1.89 \times 10^5$ vs. $13.7 \pm 2.24 \times 10^5$ /mL, (Table 1). Cell viability did not differ between cells with and without hemin exposure (99.3 ± 1.15 % vs. 99 ± 0.89 %). Expression of CD71 (transferrin receptor), which is required for iron delivery into cells, and CD235a (glycophorin a) were measured by flow cytometry. As shown in Table 2, cells were strongly positive for CD71 and rarely positive for CD235a. Treatment with 40 μ M hemin for 72 h did not change the MFI or the CD235a-positive rate, but increased the CD71-positive rate, as seen in previous studies in K562 cells [17, 23–25].

To determine whether hemin-induced differentiation

was detectable at the transcriptional level, mRNAs of erythroid transcription factors were measured by quantitative real-time RT-PCR. Exposure to hemin for 48 h significantly increased α -globin, β -globin, GATA1, and NFE2 expression (Fig. 2B; $p < 0.05$, hemin: $n = 13$, control: $n = 16$), as also found previously [26–28]. Because induction of α -globin by hemin was significantly higher than that of β -globin, we used α -globin as a molecular marker for erythroid differentiation in assessment of T3 action. Since GATA2 was also elevated, along with the globin genes, GATA1 and NFE2, GATA2 mRNA upregulation was used as a marker of erythropoiesis in hemin-treated K562 cells. Hemin also significantly augmented mRNAs for TR α , TR β and RXR α .

Influence of T3 on proliferation of K562 cells

To determine whether T3 affected cell proliferation, K562 cells were incubated in the absence or presence of 1 nM or 100 nM T3 for up to 96 h and viable cells were counted. As shown in Fig. 3, T3 up to 100 nM did not alter cell proliferation for at least 96 h.

Effects of T3 on hemin-induced differentiation of K562 cells

The effect of T3 on the benzidine-positive rate in K562 cells was measured. Various concentrations of T3 and an equal amount of 0.1 N NaOH as a negative control were added to cultures 24 h prior to hemin addition. The benzidine-positive rate of hemin-treated cells after 72 h was 71% (70.25–74%), and this was not significantly changed by T3 at 1 nM (64.5% (61.25–70%)) or 100 nM (67% (59.25–68.75%)) ($p = 0.056$, $n = 4$ in each group). Hemoglobin production was analyzed after various concentrations of T3 (0.1–100 nM) and 0.1 N NaOH as a negative control were added 24 h prior to hemin treatment. Hemoglobin production per 5×10^6 cells was measured after 72 h of hemin treatment. T3 at 1 nM and 10 nM significantly augmented hemin-induced hemoglobin production, while 100 nM T3 abrogated this stimulatory effect. Maximal enhancement was detected at 1 nM T3 (Fig. 4A-1). Cell viability did not differ among hemin-treated cells exposed to various concentrations of T3, although cell proliferation with 1 nM and 10 nM T3 was significantly decreased compared to cells without T3 (Fig. 4A-2). Because 1 nM and 10 nM T3 had inducible effects on hemoglobin production, we examined whether T3 itself can stimulate hemoglobin production. However, K562 cells treated with 1 nM or 10 nM T3 for 72 h did

not have increased hemoglobin content (Fig. 4A-3).

The MFI and rate of CD71 expression were maximally and significantly enhanced in hemin-treated K562 cells with 0.1 nM T3 pre-incubation, while this stimulation was attenuated by 1–100 nM T3 (Fig. 4B). There was no significant difference in CD235a expression in hemin-treated K562 cells with or without T3 (data not shown).

Finally, mRNA expression was examined in hemin-treated K562 cells with pre-incubation with 0, 1 or 100 nM T3 for 24 h ($n = 7$ per group). Cells were collected after 48 h of hemin exposure. T3 had a significant stimulatory effect on α -globin mRNA expression at 1 nM T3 compared to control (hemin(+)), and this effect was abrogated at 100 nM T3. GATA2 expression in cells pretreated with 100 nM T3 was significantly lower than that in cells pretreated with 1 nM T3. The RXR α expression level in cells pretreated with 100 nM T3 was also significantly lower than that in cells pretreated with 1 nM T3 (Fig. 4C).

Discussion

In this study, we demonstrated that a high concentration of T3 attenuates the stimulatory effect of T3 on hemin-induced erythropoiesis at lower concentrations in K562 cells. We also showed for the first time that hemin increases mRNA for TR α , TR β , and RXR α . T3 augmented hemin-induced hemoglobin production (1 and 10 nM), CD71 expression (0.1 nM), and α -globin mRNA expression (1 nM), while this augmentation was abrogated at a higher T3 concentration of 100 nM.

Hemin induced hemoglobin production and decreased cell proliferation without cell death. T3 at a lower concentration augmented hemin-induced hemoglobin production with decreased cell proliferation without changing cell viability. T3 alone at 100 nM did not alter cell proliferation, and 1 and 10 nM T3 did not cause hemoglobin production, suggesting that 100 nM T3 was not toxic, and that 1 and 10 nM T3 did not have erythropoietic effects in this culture. Hemin also significantly decreased cell proliferation. Collectively, these data indicate that lower concentrations of T3 stimulate hemin-induced hemoglobin production, while a higher concentration of T3 abolishes this effect. Changes in CD71 and α -globin mRNA expression with various concentrations of T3 during hemin-induced erythropoiesis were consistent with the changes in hemoglobin production. However, the benzidine-positive rate

Fig.2 Changes in K562 cells after exposure to hemin.

(A) Hemoglobin content increased in a dose-dependent manner after exposure to various concentrations of hemin for 72 h. Experiments were performed in triplicate in different dishes. * $p < 0.05$ by Kruskal-Wallis test. (B) Changes in mRNA expression in K562 cells during hemin-induced erythropoiesis were measured for transcription factors related to erythropoiesis (B-1: GATA1, B-2: GATA2, B-3: NFE2, B-4: α -globin, B-5: β -globin) and for thyroid hormone receptors (B-7: TR α , B-8: TR β) and retinoid X receptor (B-6: RXR α). Each relative mRNA level was normalized based on the level for PPIA. Differences in mRNA levels between control and hemin-treated cells were analyzed by Mann-Whitney U test (* $p < 0.05$, hemin: $n = 13$, control: $n = 16$). Hemin treatment was performed at 40 μM for 48 h.

Table 1 Cell proliferation and cell viability of K562 cells in the presence or absence of hemin at each indicated time point.

	Calculated time point	Hemin(-)	Hemin(+)	
Cell count ($\times 10^5$ /mL)	0 hour	2		
	72 hours	13.7 ± 2.24	6.72 ± 1.89	$p < 0.05$
Cell viability (%)	0 hour	99		
	72 hours	99.0 ± 0.89	99.3 ± 1.15	N.S.

Significance was analyzed by Mann-Whitney U test. Experiments were performed four times in individual cell cultures.

Table 2 FACS analysis of CD71 and CD235a expression with or without hemin treatment.

		Hemin(-)	Hemin(+)	
CD71	MFI	2.624 ± 0.617	4.836 ± 1.755	$p < 0.05$
	Positive rate (%)	32.7 ± 2.12	62.0 ± 1.72	$p < 0.05$
CD235a	MFI	0.334 ± 0.017	0.851 ± 0.701	N.S.
	Positive rate (%)	3.25 ± 2.94	1.35 ± 0.48	N.S.

CD71 (transferrin receptor) and CD235a (glycophorin A) were analyzed after exposure of K562 cells to hemin for 72 h. Expression levels are given as the mean fluorescence intensity (MFI) (mean $\pm$ SD) and positive rate (mean $\pm$ SD, %). Five samples in different dishes were measured. Significance was analyzed by Mann-Whitney U test.

Fig. 3 Cell proliferation analysis in K562 cells in the presence or absence of hemin.

Viable cell numbers in the indicated culture condition were calculated. T3 at 1 nM and 100 nM did not alter cell proliferation up to four days. * $p < 0.05$ by Kruskal-Wallis test. Experiments were performed four times.

Fig. 4 Effects of T3 on parameters of hemin-induced erythropoiesis.

(A-1) Hemoglobin production per 5×10^6 cells. K562 cells were incubated with various concentration of T3 for 24 h prior to hemin exposure and then counted and measured. Pre-incubation with 1 or 10 nM T3 significantly enhanced hemin-induced hemoglobin production compared to hemin-treated cells without T3 pre-incubation. $*p < 0.05$ by Kruskal-Wallis test ($n = 10$). Experiments were performed in different cultures. Hemin treatment was performed at 40μ M for 72 h. (A-2) Cell counts and viabilities in the A-1 cultures. The numbers of viable cells and cell viability in the indicated A-1 culture were calculated with an automated cell counter using trypan blue exclusion. (A-3) Hemoglobin content per 5×10^6 cells. K562 cells were incubated with various concentration of T3 for 24 h and then counted and measured. Pre-incubation with 1 or 10 nM T3 did not alter hemoglobin production compared to control (no treatment). $*p < 0.05$ by Kruskal-Wallis test (each $n = 4$). Experiments were performed in different cultures. Hemin treatment (positive control) was performed at 40μ M for 72 h. (B) FACS analysis of CD71 expression. Incubation with 0-100 nM T3 for 24 h prior to hemin treatment showed biphasic effects on MFI and the CD71-positive rate. Error bars for MFI show mean $\pm$ SD (each $n = 5$). $*p < 0.05$ by Kruskal-Wallis test. Hemin treatment was performed at 40μ M for 72 h. (C) mRNA expression. Cells were cultured with 0, 1 or 100 nM T3 for 24 h prior to hemin exposure. Hemin treatment was performed at 40μ M for 48 h. Each mRNA was normalized using the level for PPIA. Calibration of hemin (-) mRNA was set in one sample at 0 days. Transcription factors related to erythropoiesis (GATA2, NFE2, α -globin) and thyroid hormone receptors (TR α , TR β) and the partner retinoid X receptor α (RXR α) were measured. $*p < 0.05$ by Kruskal-Wallis test ($n = 7$).

did not differ between T3-pretreated and control cells after hemin induction. This is probably because benzidine positivity with hemin treatment may be affected by free hemin diffusing into the cells, which may cause misleading results [25, 29]. Overall, these findings indicate that T3 at a lower concentration acts as an enhancer of hemin and that 100 nM T3 blocks this effect in our model of erythroid differentiation.

A biphasic action of T3 has been reported in colony growth of K562 cells and proliferation of erythroid burst forming units (BFU-Es) in human peripheral blood and bone marrow mononuclear cells in serum free culture [30, 31]. A recent report showed that exposure of human hematopoietic progenitor cells to higher or lower than the normal concentration of thyroid hormone had a significant influence on clonogenicity and induced apoptosis [32]. Moreover, strong inhibition of terminal differentiation by T3 has been reported in Epo responsive NFS-60 cells [33] and we previously showed that T3 suppresses activin-induced K562 differentiation [19]. Hematopoietic progenitor cells obtained from hyperthyroid patients exhibit increased proliferative potential of BFU-Es and upregulation of cell cycle markers, compared to those from healthy volunteers [34]. These studies suggest that T3 exerts classical augmentation, but also has a suppressive effect on erythropoiesis, especially at a high concentration. These different responses to T3 could be due to 'stage-specific effects' of T3 on proliferation or differentiation, as proposed by Ingley *et al.*, in which T3 stimulates proliferation of immature erythroid cells and inhibits maturation promoted by Epo [35, 36]. Our findings of blockage of enhancement of erythropoiesis by 100 nM T3 in hemin-induced K562 cells without a toxic effect may reflect the action of T3 on hemoglobin synthesis because hemin is a strong inducer of heme biosynthesis. Regarding the relationship between thyrotoxicosis and erythropoiesis, clinical reports showing that erythroid hyperplasia occurs in hyperthyroidism have not accounted for the level of hyperthyroidism [5]. Treatment of hyperthyroidism has also been found to restore hemoglobin levels in GD with anemia [6].

Hemin induced increases in GATA1, GATA2, NFE2 mRNA levels in K562 cells, along with α -globin and β -globin upregulation. GATA1 is highly expressed in erythroid cells and regulates expression of many erythroid genes, including genes for globins and heme synthesis enzymes [37–39]. GATA2 is usually expressed in early erythroid precursors and determines cell lin-

eage throughout hematopoiesis, before being down-regulated in the final stage of erythropoiesis [37, 40]. NFE2, which regulates erythroid maturation and differentiation, is required for activation of the locus control region of the globin gene cluster during erythroid cell differentiation [41]. The increased GATA1 and NFE2 levels induced by hemin in K562 cells are in accord with previous findings [26], but the significance of GATA2 elevation after hemin exposure in K562 cells is unclear.

T3 at 100 nM significantly decreased GATA2 mRNA expression compared to 1 nM T3 in hemin-induced K562 cells, suggesting an association of GATA2 mRNA and T3 during erythropoiesis [19]. Expression of RXR α mRNA in cells treated with 100 nM T3 was also decreased compared to that with 1 nM T3 in hemin-induced K562 cells. A further study is needed to explore the roles of GATA2 and RXR α in T3 action during erythropoiesis. Our results also provide the first evidence for upregulation of TR α , TR β , and RXR α mRNA in hemin-treated K562 cells. There has been no previous report of thyroid hormone receptor upregulation during erythroid differentiation.

A major limitation of the study is that the effects of T3 on erythropoiesis were detected using a single model based on hemin-treated K562 cells. To confirm the basic effect of T3 on erythropoiesis, further studies using different cell lines and different erythropoietic agents are needed. Similarly, upregulation of TRs during erythropoiesis was only detected in hemin-treated K562 cells, and a further study is required to determine how TRs are related to erythropoiesis. A second limitation is that there are no single parameter showed upregulation at a low concentration of T3 and down-regulation at a high concentration of T3. Hemoglobin production with various concentrations of T3 was closely related to reduced cell proliferation in our culture system, and cell cycle-regulated mRNAs have been reported to change during hemin-induced erythropoiesis [42]. Therefore, transcription factors related to the cell cycle may be candidates for exploration in a further study of the underlying mechanism.

Conclusion

We conclude that a higher concentration of T3 attenuates the stimulatory effect on hemin-induced erythroid differentiation without exerting a toxic effect in K562 cells. This conclusion is compatible with the well-

known association between thyroid hormone and Hb levels in euthyroid to thyrotoxic GD patients. TR α , TR β and RXR α mRNAs were induced by hemin and GATA2 and RXR α mRNA were altered synchronously with various concentrations of T3 during hemin-induced differentiation, suggesting the importance of these factors in this T3 activity in hemin-treated K562 cells.

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Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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