Biochemical and Biophysical Research Communications 454 (2014) 102–108

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Effect of 5-aminolevulinic acid on erythropoiesis: A preclinical *in vitro* characterization for the treatment of congenital sideroblastic anemia

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ARTICLE INFO

Article history:
Received 28 September 2014
Available online 18 October 2014

Keywords:
5-Aminolevulinic acid
Erythroid cells
Heme
5-Aminolevulinic acid synthase 2
Congenital sideroblastic anemia

ABSTRACT

Congenital sideroblastic anemia (CSA) is a hereditary disorder characterized by microcytic anemia and bone marrow sideroblasts. The most common form of CSA is attributed to mutations in the X-linked gene *5-aminolevulinic acid synthase 2* (*ALAS2*). ALAS2 is a mitochondrial enzyme, which utilizes glycine and succinyl-CoA to form 5-aminolevulinic acid (ALA), a crucial precursor in heme synthesis. Therefore, ALA supplementation could be an effective therapeutic strategy to restore heme synthesis in CSA caused by *ALAS2* defects. In a preclinical study, we examined the effects of ALA in human erythroid cells, including K562 cells and human induced pluripotent stem cell-derived erythroid progenitor (HiDEP) cells. ALA treatment resulted in significant dose-dependent accumulation of heme in the K562 cell line. Concomitantly, the treatment substantially induced erythroid differentiation as assessed using benzidine staining. Quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis confirmed significant upregulation of heme-regulated genes, such as the globin genes [hemoglobin alpha (*HBA*) and hemoglobin gamma (*HBG*)] and the heme oxygenase 1 (*HMOX1*) gene, in K562 cells. Next, to investigate the mechanism by which ALA is transported into erythroid cells, quantitative RT-PCR analysis was performed on previously identified ALA transporters, including *solute carrier family 15 (oligopeptide transporter), member (SLC15A) 1*, *SLC15A2*, *solute carrier family 36 (proton/amino acid symporter), member (SLC36A1)*, and *solute carrier family 6 (neurotransmitter transporter), member 13 (SLC6A13)*. Our analysis revealed that *SLC36A1* was abundantly expressed in erythroid cells. Thus, gamma-aminobutyric acid (GABA) was added to K562 cells to competitively inhibit SLC36A1-mediated transport. GABA treatment significantly impeded the ALA-mediated increase in the number of hemoglobinized cells as well as the induction of *HBG*, *HBA*, and *HMOX1*. Finally, small-interfering RNA-mediated knockdown of ALAS2 in HiDEP cells considerably decreased the expression of *HBA*, *HBG*, and *HMOX1*, and these expression levels were rescued with ALA treatment. In summary, ALA appears to be transported into erythroid cells mainly by SLC36A1 and is utilized to generate heme. ALA may represent a novel therapeutic option for CSA treatment, particularly for cases harboring *ALAS2* mutations.

Abbreviations: CSA, congenital sideroblastic anemia; ALA, 5-aminolevulinic acid; ALAS2, 5-aminolevulinic acid synthase 2; HBA, hemoglobin alpha; HBG, hemoglobin gamma; HMOX1, heme oxygenase 1; GABA, gamma-aminobutyric acid; HiDEP, human induced pluripotent stem cell-derived erythroid progenitor; XLSA, X-linked sideroblastic anemia; FECH, ferrochelatase; PBGD, porphobilinogen deaminase; AlaAcBu, 1-(butyryloxy)ethyl-5-amino-4-oxopentanoate; RT-PCR, reverse transcription polymerase chain reaction.

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http://dx.doi.org/10.1016/j.bbrc.2014.10.050
0006-291X/

1. Introduction

5-Aminolevulinic acid (ALA), an important precursor of heme, is a natural amino acid biosynthesized in the mitochondria of both animals and plants [1]. The compound is synthesized from glycine and succinyl-CoA in mitochondria, which is catalyzed by two different ALA synthases (ALAS): one expressed ubiquitously (ALAS1) and the other expressed only by erythroid precursors (ALAS2) [2]. During synthesis, ALA is exported to the cytosol where it is converted to coproporphyrinogen III. All the remaining steps of heme biosynthesis take place inside the mitochondria. Coproporphyrinogen III is imported into the mitochondria, and is finally catalyzed into protoporphyrin IX. Subsequently, heme is generated by the insertion of ferrous iron into protoporphyrin IX, which is catalyzed by ferrochelatase (FECH). Heme synthesis mostly occurs in developing erythroblasts located in the bone marrow to produce hemoglobin, whereas approximately 15% of the daily synthesis occurs in the liver to form heme-containing enzymes, such as cytochrome P450 [3]. Consequently, mutations in the genes involved in the heme biosynthetic pathway can cause impairment of oxygen delivery, mitochondrial respiratory chain activity, and drug metabolism, leading to the onset of porphyria and congenital sideroblastic anemia (CSA) [3,4].

CSA is a hereditary microcytic anemia characterized by bone marrow sideroblasts with excess iron deposition in the mitochondria. The most common type of CSA is X-linked sideroblastic anemia (XLSA), which is caused by defects in the X-linked gene *ALAS2* [5–7]. Most of the XLSA-associated mutations in *ALAS2* are missense substitutions resulting in loss of functionality, whereas mutations in the *ALAS2* regulatory region, such as the promoter [8] and intron 1 [9], lead to decreased *ALAS2* expression. ALAS2 missense mutations commonly decrease the binding of pyridoxal 5′-phosphate (PLP; vitamin B6), which is a cofactor for ALAS2 enzymatic activity, thus accounting for the PLP responsiveness in XLSA patients carrying such mutations [7]. However, nearly half of XLSA cases are unresponsive to PLP [5,6,9]. Therefore, ALA supplementation could be a useful alternative therapeutic strategy for restoring heme synthesis in CSA disorders caused by defects in ALAS2.

As a preclinical study, we examined the effects of ALA in human erythroid cells. Furthermore, we investigated the molecular mechanism by which ALA is transported into erythroid cells.

2. Materials and methods

2.1. Cell culture and reagents

All cells were grown in a humidified incubator at 37 °C with 5% carbon dioxide. Human K562 erythroleukemia cell lines were maintained in RPMI-1640 medium containing 10% fetal bovine serum (Biowest, Miami, FL) and 1% penicillin–streptomycin (Sigma, St. Louis, MO, USA). Human induced pluripotent stem (iPS) cell-derived erythroid progenitor (HiDEP) cell lines were cultured in StemSpan Serum-Free Expansion Medium (STEMCELL Technologies, Vancouver, BC, Canada) containing 3-U/mL erythropoietin, 1-μg/mL doxycycline (Sigma), and 1-μM dexamethasone (Sigma), as described previously [10]. ALA hydrochloride (SBI Pharmaceuticals Co., Ltd., Tokyo, Japan) and gamma-aminobutyric acid (GABA) (Sigma) were prepared with distilled water. Erythropoietin was a kind gift of Kyowa Hakko Kirin Co. Ltd. (Tokyo, Japan).

2.2. Extraction of mouse tissue RNA

Mouse heart, lung, liver, spleen, kidney, intestine, and muscle were frozen in liquid nitrogen immediately after isolation. Frozen tissues were homogenized and total RNA was isolated using TRIzol (Invitrogen, Carlsbad, CA, USA). A magnetic activated cell sorter system (Miltenyi Biotec, Auburn, CA, USA) was used to separate mouse Ter119-positive erythroblasts from the bone marrow.

2.3. Real-time quantitative reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was purified using TRIzol (Invitrogen), and 1 μg of purified total RNA was used to synthesize complementary DNA (cDNA) with ReverTra Ace qPCR RT Master Mix (TOYOBO). Reaction mixtures (20 μL) for real-time quantitative RT-PCR consisted of 2 μL of cDNA, 10 μL of Quantitect SYBR Green PCR Master Mix (QIAGEN), and appropriate primers. Product accumulation was monitored by measuring SYBR Green fluorescence and normalized relative to *GAPDH* messenger RNA (mRNA).

To evaluate expression levels of human and murine ALA transporters [i.e., solute carrier family 36 (proton/amino acid symporter), member (SLC36A1), solute carrier family 15 (oligopeptide transporter), member (SLC15A) 1, SLC15A2, and *solute carrier* family 6 (neurotransmitter transporter), *member 13* (SLC6A13)], an amplified cDNA fragment of each gene was cloned into the pGEM™-T Easy Vector (Promega, Madison, WI), and was used as an internal standard in quantitative RT-PCR. The plasmid copy number was calculated as follows: copy number (copy/μL) = $6.02 \times 10^{23} \times$ [plasmid DNA concentration (μg/μL)] $\times 10^{-6}$/[total plasmid size (base pair)] $\times$ 660. Primer sequences are available upon request.

2.4. Heme content

Heme content was determined fluorometrically as described previously [11]. In brief, cell pellets were suspended in 2-M oxalic acid and boiled (100 °C) for 30 min to dissociate protoporphyrin IX and iron from heme. Subsequently, fluorescence for protoporphyrin IX was measured at 400 nm (excitation) and 662 nm (emission). To exclude endogenous levels of protoporphyrin IX, the fluorescence based on unboiled samples were subtracted.

2.5. Assay of erythroid differentiation of K562 cells

Erythroid differentiation of K562 cells was scored by benzidine staining as described previously [12]. Benzidine (o-dianisidine) was obtained from Sigma. Benzidine-positive cells were quantified by light microscopy (n = 600). Viable cells were counted by trypan blue dye (Invitrogen) exclusion.

2.6. Microarray analysis

SurePrint G3 Human GE 8 × 60 K Microarrays (G4851B) (Agilent, Palo Alto, CA, USA) and Human Oligo chip 25 k (Toray, Tokyo, Japan) were used, respectively, for expression profiling of ALA-treated K562 cells and ALAS2-knockdowned HiDEP cells, as described previously [13]. Gene Ontology analysis was performed as previously described [13].

2.7. Silencing of ALAS2 gene expression by small interfering RNA (siRNA)

We used siGENOME SMARTpool (Thermo Scientific Dharmacon, Lafayette, CO) to perform siRNA-mediated transient knockdown in HiDEP cells. The antisense sequences of the siRNA for human *ALAS2* were CUAGCUGAAUUGAGCCUAA, GAUCCAAGGUAUCCGUAAC, CG

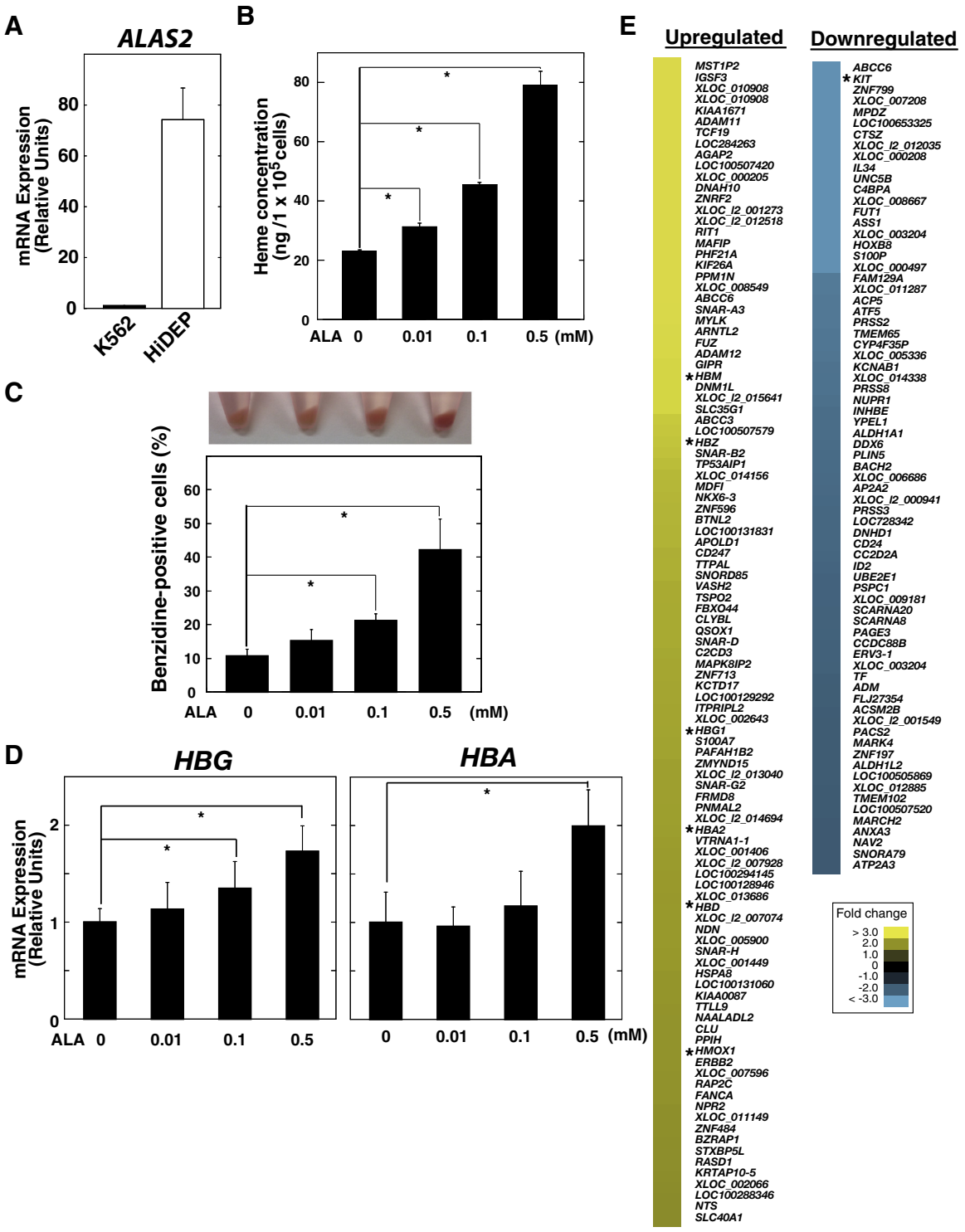

Fig. 1. ALA treatment induced erythroid differentiation of K562 cells. (A) Quantitative RT-PCR analysis of human *ALAS2* in K562 and HiDEP cells. The data are expressed as means ± standard deviation. The expression of ALAS2 in K562 cells was set to 1. (B) Heme level, (C) cell pellet and percentage of benzidine-positive cells, and (D) quantitative RT-PCR of *HBG* and *HBA* in K562 cells treated with ALA (0.01, 0.1, and 0.5 mM) for 72 h. The data are expressed as means ± standard error (SE). $n = 4$; $^{*}p < 0.05$. (E) The heat map depicts the fold change resulting from ALA treatment (0.5 mM, 72 h) in K562 cells. Genes displaying >2-fold changes are shown. Asterisk indicates globin genes, *HMOX1*, and *KIT*.

UCUGGUGUAGUAAUGAU, and GCUGAUGCAUAUCCCUUUG. siGENOME Non-Targeting siRNA Pool #1 (Thermo Scientific Dharmacon) was used as a negative control. Each siRNA was transfected into 2.5×10^6 HiDEP cells using the Amaxa Cell Line Nucleofector Kit V with the T-016 program (Amaxa Biosystems, Cologne, Germany), and the cells were harvested at 48 h.

2.8. Western blotting

Western blotting was conducted as described previously [13,24]. Antibodies for ALAS2 (ab184964) and Actin (I-19) were purchased from Abcam (Cambridge, UK) and Santa Curz Biotechnology (Santa Cruz, CA, USA), respectively.

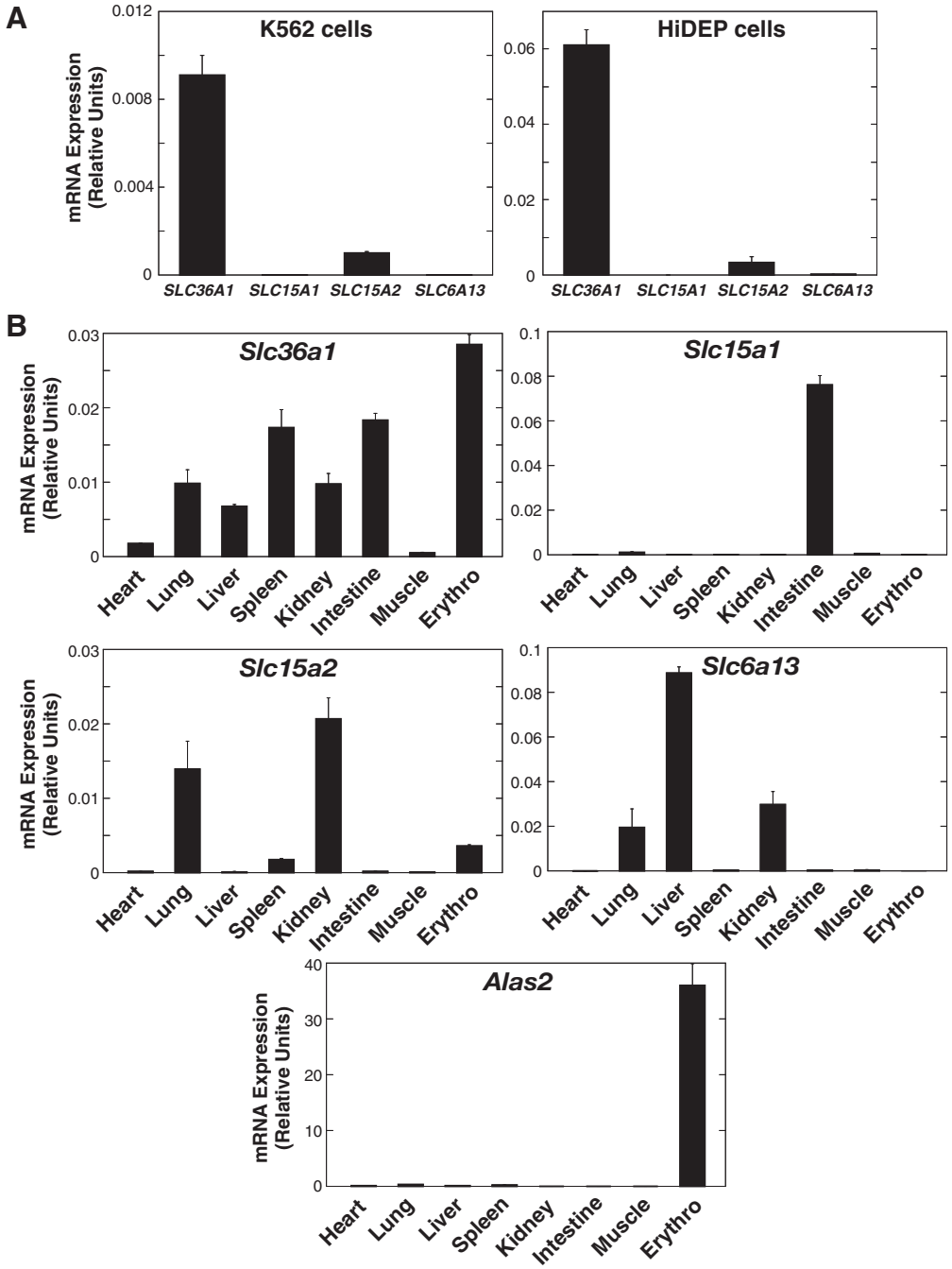

Fig. 2. SLC36A1 mRNA is abundantly expressed in erythroid cells. (A) Quantitative RT-PCR analysis of human *SLC36A1*, *SLC15A1*, *SLC15A2*, and *SLC6A13* in K562 and HiDEP cells. The copy number of each gene relative to that of *GAPDH* was calculated (n = 3, mean ± SE). (B) Quantitative RT-PCR analysis of murine *Slc36a1*, *Slc15a1*, *Slc15a2*, *Slc6a13*, and *Alas2* in various mouse tissues. The copy number of each gene relative to that of *GAPDH* was calculated (n = 3, mean ± SE).

2.9. Statistics

Statistical significance was assessed by two-sided Student's t test. In all analyses, a p value <0.05 was considered to indicate statistical significance.

3. Results and discussion

3.1. ALA treatment induces erythroid differentiation in K562 cells

First, we assessed the effects of ALA in the K562 erythroid cell line. The expression of *ALAS2* in K562 cells was remarkably lower than those in HiDEP cells (76-fold, Fig. 1A). Thus, K562 cells appear to exhibit reduced hemoglobinization due to insufficient heme supply, as suggested previously based on human erythroid cell line (YN-1) [14]. As expected, ALA treatment stimulates a significant augmentation in intracellular heme level in K562 cells, in a dose-dependent manner (Fig. 1B). Concomitantly, ALA substantially induces erythroid differentiation as evidenced by a reddish pellet (Fig. 1C, upper) and increased the percentage of benzidine-positive cells (Fig. 1C, lower), which reflects the peroxidase activity of hemoglobin [11]. Besides its role as a prosthetic group of hemoproteins, as represented by hemoglobin production in erythroid cells, heme is also involved in the transcriptional regulation of *heme oxygenase 1* (*HMOX1*) [15] and globin genes [16,17] through

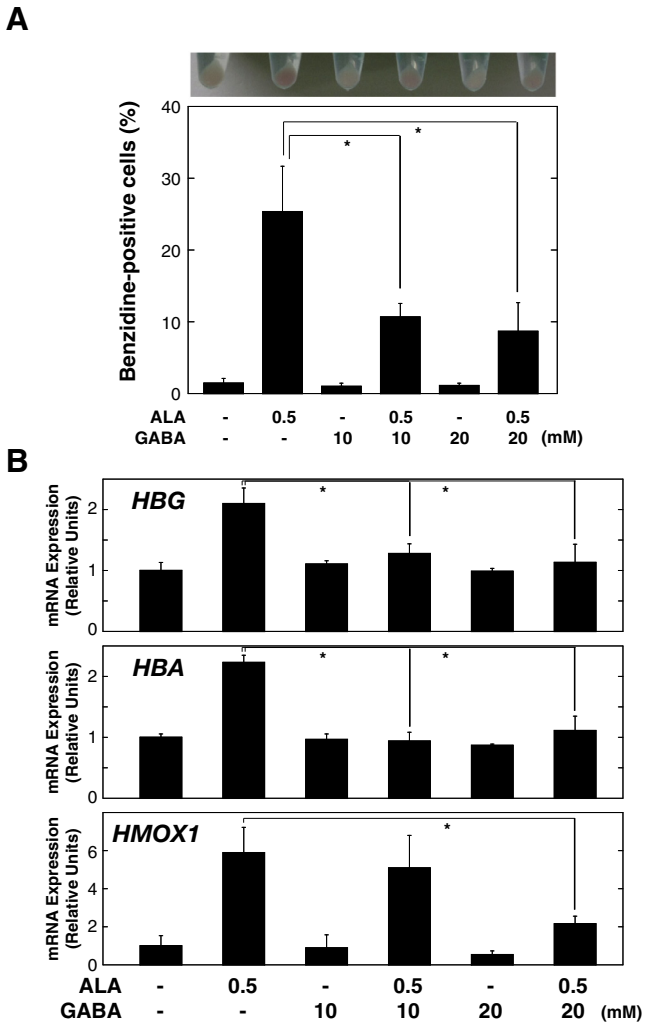

Fig. 3. GABA treatment significantly impedes the effects of ALA in K562 cells. (A) Cell pellet and percentage of benzidine-positive cells, and (B) quantitative RT-PCR of *HBG*, *HBA*, and *HMOX1* (B) in K562 cells treated with ALA (0.5 mM) ± GABA (10 or 20 mM) for 72 h. The data are expressed as means ± standard deviation ($n = 4$). $^{*}p < 0.05$.

transcriptional corepressor BTB and CNC homology 1 (BACH1). Quantitative RT-PCR demonstrated significant upregulation of *HBA*, *HBG*, and *HMOX1* with ALA treatment (Figs. 1D and 3B). In addition, expression profiles of K562 cells treated with 0.5-mM ALA confirmed the upregulation of various globin genes (*hemoglobin mu* (*HBM*), *hemoglobin zeta* (*HBZ*), *HBG*, *HBA*, and *hemoglobin delta* (*HBD*)) as well as *HMOX1*, whereas other erythroid-related genes were not observed (Fig. 1E and Supplementary Table 1). On the other hand, we found the downregulation of *KIT*, presumably reflecting erythroid differentiation [18].

K562 cells have been widely used as a model of erythroid differentiation induced by hemin [19]; however, the effects of ALA on K562 differentiation have not been examined in detail. A previous report by Berkovitch-Luria et al. suggested that a multifunctional ALA derivate (1-(butyryloxy)ethyl-5-amino-4-oxopentanoate (AlaAcBu)), which undergoes metabolic hydrolysis to yield ALA and butyric acid (a histone deacetylase inhibitor), induced erythroid differentiation of K562 cells [20]. In addition to promoting heme synthesis and globin gene induction, AlaAcBu also activated key enzymes in the heme biosynthesis pathway, such as porphobilinogen deaminase (PBGD) and FECH [20]. Conversely, we did not observe significant upregulation of heme biosynthetic enzymes with ALA treatment (Supplementary Table 1). Therefore, our findings provide insight into the multiple effects of AlaAcBu, and we propose that increases in PBGD and FECH expression are mainly mediated through the inhibition of histone deacetylase activity by butyric acid.

3.2. SLC36A1 is abundantly expressed in erythroid cells

The molecular mechanisms underlying the ALA transportation into erythroid cells remain unknown. Thus, we performed quantitative RT-PCR analysis of previously identified ALA transporters. We focused on two proton-coupled oligopeptide transporters (SLC15A1: peptide transporter (PepT) 1 and SLC15A2: PepT2) [21,22], the human proton-coupled amid acid transporter (SLC36A1), [23] and a beta transporter (SLC6A13: GABA transporter 2) [24]. Fig. 2A shows that *SLC36A1* was abundantly expressed in both K562 and HiDEP cells, whereas the expression levels of *SLC15A1* and *SLC6A13* were low to almost undetectable in erythroid cells. To further confirm the data, we also performed quantitative RT-PCR analysis in various mouse tissues, including Ter119-positive erythroblasts, which was validated by analyzing *Alas2* expression (Fig. 2B, bottom). Mouse *Slc36a1* was abundantly expressed in erythroblasts, although it also detected in other tissues, including the intestine, spleen, lung, kidney, and liver (Fig. 2B). Similar to the analyses performed in K562 and HiDEP cells (Fig. 2A), murine *Slc15a2* was also weakly expressed, but *Slc15a1* and *Slc6a13* were almost undetectable in erythroid cells (Fig. 2B). We also confirmed that *Slc15a1* was almost exclusively expressed in intestine, as suggested previously [22]. Low expression of *SLC15A1* and *SLC15A2* in K562 cells is also consistent with an earlier study by Ahlin et al. [21]. In addition, the online gene annotation database BioGPS (BioGPS: http://biogps.org/#goto=welcome) reports that *SLC36A1* is abundantly expressed in CD71-positive erythroblasts (Supplementary Fig. 1). To date, there has been no reports focusing on the transcriptional regulation of *SLC36A1* in erythroid cells. Transcription factor GATA-1 has been reported to regulate the expression of key erythroid-related genes [25]. Interestingly, recent genome-wide analysis of GATA-1 chromatin occupancy based on the coupling of next-generation DNA sequencing technology with chromatin immunoprecipitation sequencing showed a GATA-1 peak in the *SLC36A1* locus [25]. Thus, GATA-1 might be involved in the transcriptional activation of *SLC36A1* in erythroblasts.

3.3. GABA treatment significantly impedes the effects of ALA in K562 cells

ALA and GABA are structurally similar; therefore, both compounds could serve as substrates for SLC36A1 and SLC15A1 [23,26]. On the basis of this logic, we used GABA as a competitive inhibitor of ALA transportation into K562 cells, as reported previously [23]. Fig. 3 shows that ALA-mediated increase in the number of hemoglobinized cells as well as the induction of *HBA*, *HBG*, and *HMOX1* were significantly obstructed with GABA treatment. Because the expression of *SLC15A1* was negligible in comparison with *SLC36A1* (Fig. 2A), we hypothesized that the transport of ALA into K562 cells is primarily mediated by SLC36A1. Therefore, we performed siRNA-mediated knockdown of *SLC36A1*, *SLC15A2*, or both (*SLC36A1* and *SLC15A2*) in the K562 cell line. However, knockdown of the genes did not significantly inhibit ALA-mediated increase of hemoglobinized cells and induction of *HBA*, *HBG*, and *HMOX1* (data not shown). We speculate that the knockdown efficiency might be too low and; therefore, failed to exhibit statistical significance. Alternatively, we cannot exclude the possibility that other transporters, or even passive diffusion, might contribute to the transportation of ALA into K562 cells. Further analyses are

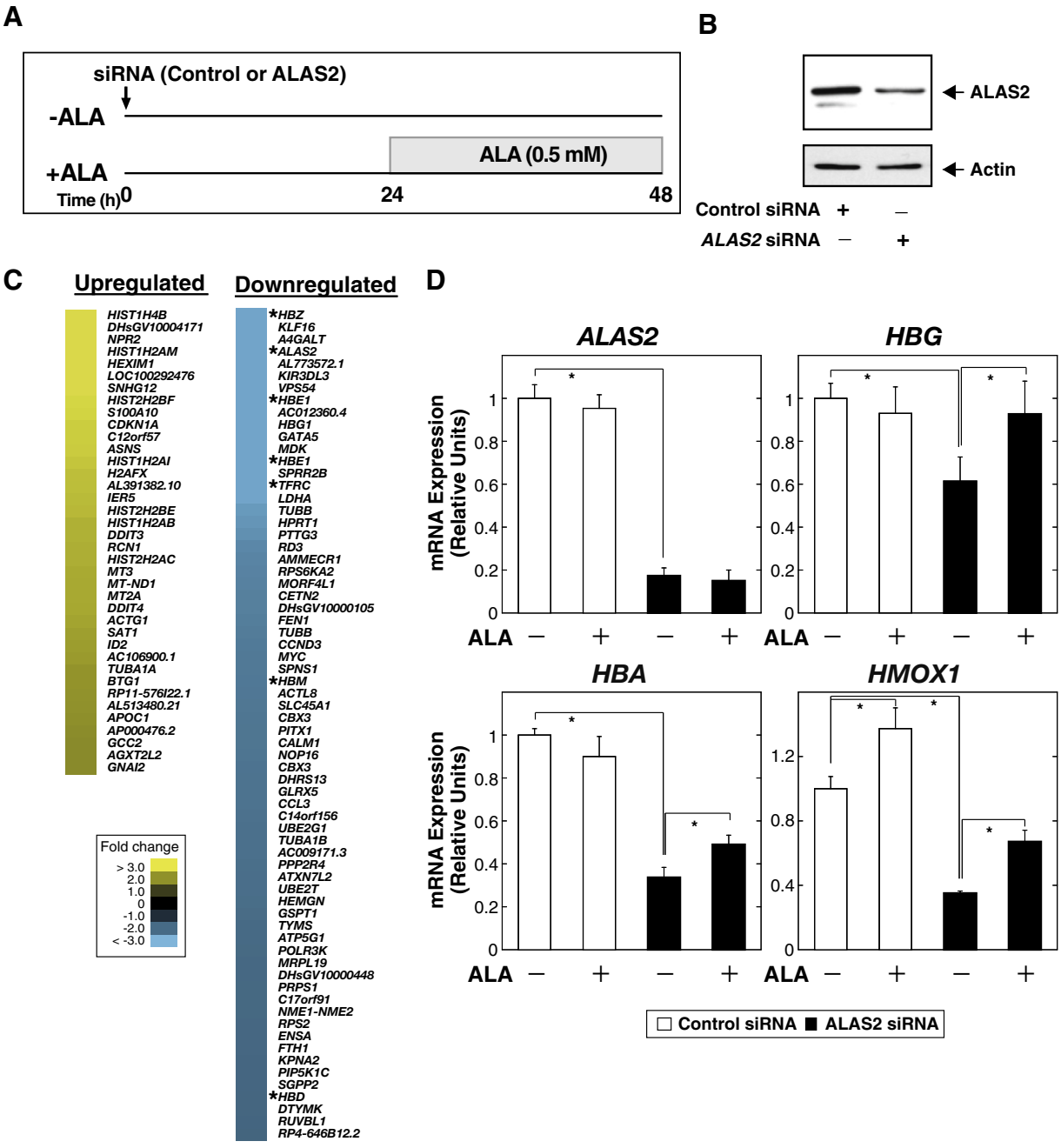

Fig. 4. ALA restores defects in ALAS2 deficiency in HiDEP cells. (A) Experimental protocol for *ALAS2* knockdown and *ALA* rescue in HiDEP cells. (B) siRNA-mediated ALAS2 knockdown in HiDEP cells. Anti-ALAS2 Western blot of whole cells extracts. Actin was a loading control. (C) The heat map depicts the fold change resulting from *ALAS2* knockdown in HiDEP cells. Genes displaying >2-fold changes are shown. Asterisk indicates globin genes, *ALAS2*, and *TFRC*. (D) Quantitative RT-PCR analysis of *ALAS2*, *HBG*, *HBA*, and *HMOX1* in HiDEP cells following the experimental protocol of (A). The data are expressed as means ± SE ($n = 4$). $^*p < 0.05$.

required to clarify the mechanism by which ALA is transported into erythroid cells.

3.4. ALA restores defects in ALAS2 deficiency in human iPS cell-derived erythroblasts

To recapitulate the phenotype of XLSA, we performed siRNA-mediated knockdown of *ALAS2* in HiDEP cells, which were subsequently treated with ALA (Fig. 4A). We first performed expression profiling based in *ALAS2*-knockdown HiDEP cells, which was confirmed by Western blotting (Fig. 4B). The analysis revealed >2-fold upregulation and downregulation of 38 and 68 genes caused by *ALAS2* knockdown, respectively (Fig. 4C and Supplementary Table 2). The downregulated gene ensemble included *ALAS2*, globins (*HBZ*, *HBG*, *HBE*, *HBD*, and *HBM*) in addition to genes involved in iron metabolism (ferritin heavy chain 1 and transferrin receptor (*TFRC*)). Gene ontology analysis revealed significant enrichment of cellular iron ion homeostasis ($p = 0.000076$), cell division ($p = 0.00062$), DNA repair ($p = 0.0006$), and translation ($p = 0.018$), implying that heme was involved in various biological processes in erythroid cells. The downregulation of *TFRC* may imply iron overload due to decreased porphyrin synthesis, which diminishes *TFRC* mRNA stability through the inactivation of iron regulatory proteins [27]. However, ringed sideroblasts were not observed in HiDEP cells with *ALAS2* knockdown (data not shown). Noticeably, ALA treatment significantly improved the effects of *ALAS2*

knockdown-mediated downregulation of *HBA*, *HBG*, and *HMOX1* (Fig. 4D), suggesting that ALA could restore defects of ALAS2 depletion in human erythroblasts.

Ideally, it would have been desirable to test the effects of ALA in a mice model of XLSA. Unfortunately, the mice are not available for further testing, because *Alas2*-knockout mice die by day 11.5 *in utero* [28]. In addition, although ringed sideroblast formation is observed after transgenic rescue of *ALAS2* in *Alas2*-knockout mice, the mice die soon after birth [29]. Thus, there is a need for the development of a CSA mouse model. Nevertheless, ALA is an endogenous amino acid that has been shown to be safe in clinical settings [1]; therefore, it could be administered in patients with XLSA who are refractory to PLP supplementation. In conclusion, our data suggests that ALA could serve as a novel therapeutic option for CSA with *ALAS2* mutations.

Acknowledgments

We are grateful to Drs. Masahiro Ishizuka, Takeo Kohda, and Kyoko Imamura (SBI Pharmaceuticals Co., Ltd.) for helpful discussion. Drs. Fujiwara and Harigae have received a research grant from Chugai Pharmaceutical Co., Ltd. The other authors declared no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.10.050.

References

[1] M. Ishizuka, F. Abe, Y. Sano, et al., Novel development of 5-aminolevurinic acid (ALA) in cancer diagnoses and therapy, Int. Immunopharmacol. 11 (2011) 358–365.
[2] K. Furuyama, K. Kaneko, P.D. Vargas, Heme as a magnificent molecule with multiple missions: heme determines its own fate and governs cellular homeostasis, Tohoku J. Exp. Med. 213 (2007) 1–16.
[3] R.S. Ajioka, J.D. Phillips, J.P. Kushner, Biosynthesis of heme in mammals, Biochim. Biophys. Acta 1763 (2006) 723–736.
[4] H.A. Dailey, P.N. Meissner, Erythroid heme biosynthesis and its disorders, Cold Spring Harb. Perspect. Med. 3 (2013) a011676.
[5] R. Ohba, K. Furuyama, K. Yoshida, et al., Clinical and genetic characteristics of congenital sideroblastic anemia: comparison with myelodysplastic syndrome with ring sideroblast (MDS-RS), Ann. Hematol. 92 (2013) 1–9.
[6] H. Harigae, K. Furuyama, Hereditary sideroblastic anemia: pathophysiology and gene mutations, Int. J. Hematol. 92 (2010) 425–431.
[7] A.K. Bergmann, D.R. Campagna, E.M. McLoughlin, et al., Systematic molecular genetic analysis of congenital sideroblastic anemia: evidence for genetic heterogeneity and identification of novel mutations, Pediatr. Blood Cancer 54 (2010) 273–278.
[8] S. Bekri, A. May, P.D. Cotter, et al., A promoter mutation in the erythroid-specific 5-aminolevulinate synthase (ALAS2) gene causes X-linked sideroblastic anemia, Blood 102 (2003) 698–704.
[9] K. Kaneko, K. Furuyama, T. Fujiwara, et al., Identification of a novel erythroid-specific enhancer for the ALAS2 gene and its loss-of-function mutation which is associated with congenital sideroblastic anemia, Haematologica 99 (2014) 252–261.
[10] R. Kurita, N. Suda, K. Sudo, et al., Establishment of immortalized human erythroid progenitor cell lines able to produce enucleated red blood cells, PLoS ONE 8 (2013) e59890.
[11] T. Fujiwara, H. Harigae, S. Takahashi, et al., Differential gene expression profiling between wild-type and ALAS2-null erythroblasts: identification of novel heme-regulated genes, Biochem. Biophys. Res. Commun. 340 (2006) 105–110.
[12] M.C. Cooper, J. Levy, L.N. Cantor, et al., The effect of erythropoietin on colonial growth of erythroid precursor cells in vitro, Proc. Natl. Acad. Sci. U.S.A. 71 (1974) 1677–1680.
[13] T. Fujiwara, H. Saitoh, A. Inoue, et al., 3-Deazaneplanocin A (DZNep), an inhibitor of S-adenosylmethionine-dependent methyltransferase, promotes erythroid differentiation, J. Biol. Chem. 289 (2014) 8121–8134.
[14] T. Nagai, H. Harigae, K. Furuyama, et al., 5-Aminolevulinate synthase expression and hemoglobin synthesis in a human myelogenous leukemia cell line, J. Biochem. 121 (1997) 487–495.
[15] J. Sun, M. Brand, Y. Zenke, et al., Heme regulates the dynamic exchange of Bach1 and NF-E2-related factors in the Maf transcription factor network, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 1461–1466.
[16] T. Tahara, J. Sun, K. Igarashi, et al., Heme-dependent up-regulation of the alpha-globin gene expression by transcriptional repressor Bach1 in erythroid cells, Biochem. Biophys. Res. Commun. 324 (2004) 77–85.
[17] T. Tahara, J. Sun, K. Nakanishi, et al., Heme positively regulates the expression of beta-globin at the locus control region via the transcriptional factor Bach1 in erythroid cells, J. Biol. Chem. 279 (2004) 5480–5487.
[18] K. Ogawa, M. Tashima, T. Toi, et al., Inhibition of erythroid differentiation by stem cell factor in K562 cells expressing the c-kit gene, Exp. Hematol. 22 (1994) 45–51.
[19] T. Rutherford, J.B. Clegg, D.R. Higgs, et al., Embryonic erythroid differentiation in the human leukemic cell line K562, Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 348–352.
[20] G. Berkovitch-Luria, S. Yakobovitch, M. Weitman, et al., A multifunctional 5-aminolevulinic acid derivative induces erythroid differentiation of K562 human erythroleukemic cells, Eur. J. Pharm. Sci. 47 (2012) 206–214.
[21] G. Ahlin, C. Hilgendorf, J. Karlsson, et al., Endogenous gene and protein expression of drug-transporting proteins in cell lines routinely used in drug discovery programs, Drug Metab. Dispos. 37 (2009) 2275–2283.
[22] S.A. Ingersoll, S. Ayyadurai, M.A. Charania, et al., The role and pathophysiological relevance of membrane transporter PepT1 in intestinal inflammation and inflammatory bowel disease, Am. J. Physiol. Gastrointest. Liver Physiol. 302 (2012) G484–G492.
[23] S. Frølund, O.C. Marquez, M. Larsen, et al., Delta-aminolevulinic acid is a substrate for the amino acid transporter SLC36A1 (hPAT1), Br. J. Pharmacol. 159 (2010) 1339–1353.
[24] M. Bermúdez Moretti, S. Correa García, C. Perotti, et al., Delta-Aminolevulinic acid transport in murine mammary adenocarcinoma cells is mediated by beta transporters, Br. J. Cancer 87 (2002) 471–474.
[25] T. Fujiwara, H. O'Geen, S. Keles, et al., Discovering hematopoietic mechanisms through genome-wide analysis of GATA factor chromatin occupancy, Mol. Cell 36 (2009) 667–681.
[26] D.T. Thwaites, L. Basterfield, P.M. McCleave, et al., Gamma-aminobutyric acid (GABA) transport across human intestinal epithelial (Caco-2) cell monolayers, Br. J. Pharmacol. 129 (2000) 457–464.
[27] S. Taketani, Acquisition, mobilization and utilization of cellular iron and heme: endless findings and growing evidence of tight regulation, Tohoku J. Exp. Med. 205 (2005) 297–318.
[28] O. Nakajima, S. Takahashi, H. Harigae, et al., Heme deficiency in erythroid lineage causes differentiation arrest and cytoplasmic iron overload, EMBO J. 18 (1999) 6282–6289.
[29] O. Nakajima, S. Okano, H. Harada, et al., Transgenic rescue of erythroid 5-aminolevulinate synthase-deficient mice results in the formation of ring sideroblasts and siderocytes, Genes Cells 11 (2006) 685–700.