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PROGRESS IN HEMATOLOGY

Iron metabolism and the related diseases

Iron metabolism in erythroid cells and patients with congenital sideroblastic anemia

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Abstract
Sideroblastic anemias are anemic disorders characterized by the presence of ring sideroblasts in a patient's bone marrow. These disorders are typically divided into two types, congenital or acquired sideroblastic anemia. Recently, several genes were reported as responsible for congenital sideroblastic anemia; however, the relationship between the function of the gene products and ring sideroblasts is largely unclear. In this review article, we will focus on the iron metabolism in erythroid cells as well as in patients with congenital sideroblastic anemia.

Keywords Heme · Iron–sulfur cluster · Congenital sideroblastic anemia · Ring sideroblasts

Iron trafficking in erythroid cells

Iron trafficking in erythroid cells is summarized in Fig. 1a, b. A transferrin receptor 1 (TFR1) on the plasma membrane binds two molecules of transferrin, each of which is capable of transporting two ferric irons. After the binding of transferrin to TFR1, the plasma membrane forms an endosome to incorporate transferrin-bound TFR1 inside of the cells. On the endosomal membrane, vacuolar type H^+-ATPase (V-ATPase) transfers protons into the endosome to acidify the inside of vesicle. Under the acidic condition, transferrin releases ferric irons (Fe^{3+}), which are subsequently reduced by the ferric reductase, six-transmembrane epithelial antigen of the prostate 3 (STEAP3), to ferrous iron (Fe^{2+}). Then, the divalent metal transporter 1 (DMT1) transports ferrous iron across the endosomal membrane into the cytoplasm. In the cytoplasm, iron could be used in several reactions as a prosthetic group of several proteins, stored in cytosolic ferritin or further transported to mitochondria for heme production or iron–sulfur cluster (ISC) formation. Since iron produces reactive oxygen species (ROS) through Fenton's reaction, excess iron is stored in ferritin as ferric iron [1]. The mechanisms involved in intracellular iron transport are largely unknown. Studies have reported that poly(rC)-binding protein 1 (PCBP1) or PCBP2 could act as an iron chaperone in eukaryotic cells, and these proteins are involved in iron transfer to cytosolic ferritin [2] or the transportation of iron from DMT1 to the iron export protein, ferroportin 1 [3], respectively. However, it is still unclear whether these PCBPs are involved in iron handling in the cytoplasm of erythroid cells.

Although the specific molecules involving cytoplasmic iron transfer from endosomes to mitochondria have not yet been identified, Richardson et al. [1] proposed the "kiss-and-run" hypothesis (Fig. 1a), suggesting that iron-containing endosomes could transiently associate with mitochondria and provide iron to mitochondria directly. Recently, Hamdi et al. [4] further provided evidence that the "kiss-and-run" mechanism is also active for effective intracellular iron supply to mitochondria in erythroid cells. This hypothesis is interesting; however, specific molecules involved in this machinery have not yet been identified.

For the import of iron into mitochondria in eukaryotic cells, SLC25A37 (mitoferrin-1) and SLC25A28 (mitoferrin-2) are essential [5, 6], and it was suggested that mitoferrin-1 plays a central role in erythroid cells during its differentiation [5]. Notably, it is confirmed that mitoferrin-1 forms a complex with ABCB10 protein [7] and ferrochelatase [8] at the inner membrane of erythroid mitochondria.

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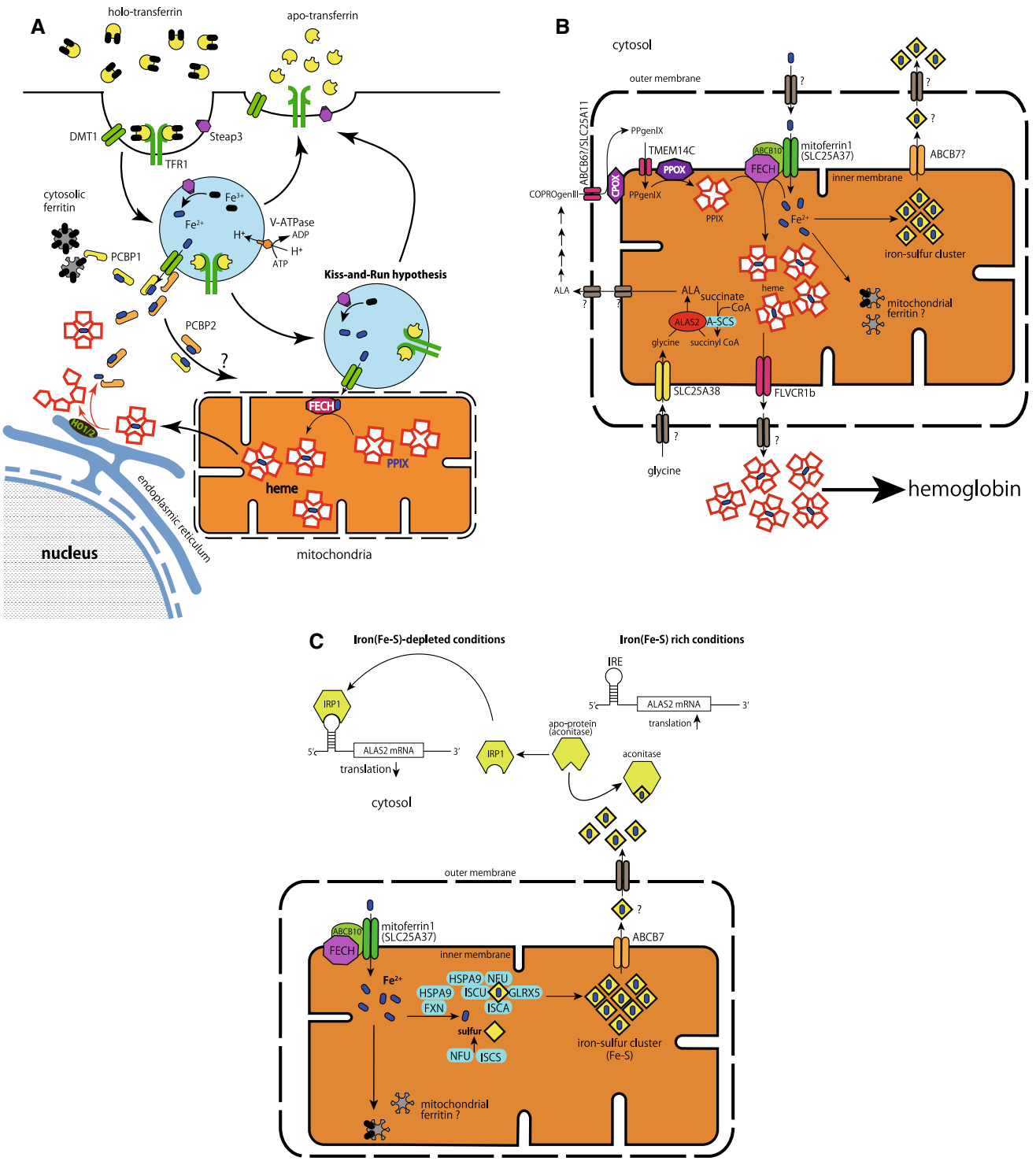

Fig. 1 Iron metabolism in erythroid cells. **a** Iron trafficking in erythroid cells. **b** Iron usage in mitochondria. **c** Iron–sulfur cluster biogenesis and translational regulation of ALAS2 expression. For the abbreviation of each protein, please refer to the text

ABCB10 (formerly known as ABC-me) is an ATP-binding cassette transporter at mitochondrial inner membrane, and it has been reported that the expression of this protein is highly induced by the transcription factor GATA-1 during erythroid differentiation [9]. ABCB10 stabilizes mitoferrin-1 to enhance its function during erythroid differentiation [7], and may support the effective utilization of iron for heme formation by ferrochelatase in erythroid cells. Indeed,

targeted disruption of Abcb10 gene in mice resulted in the embryonic lethal phenotype because of anemia [10], and the conditional knockout in a hematopoietic lineage caused anemia, with the accumulation of both protoporphyrin IX (PPIX) and iron in erythroid cells, suggesting that ABCB10 plays an important role in heme synthesis in erythroid cells [11].

The role of the iron–sulfur cluster and its biogenesis in erythroid cells

The iron–sulfur cluster (ISC) facilitates multiple functions, including redox reaction and enzymatic activity, as a prosthetic group of several proteins in virtually all cells. In eukaryotic cells, ISC is synthesized in mitochondria and cytosol, although the de novo synthesis of ISC is initiated only in mitochondria [12]. It was suggested that approximately 30 proteins are involved in the machinery [13]. In human mitochondria, proteins including ISCU, NFU, ISCA and GLRX5 provide the scaffold for assembling the ISC; the complex of ISCS and ISD11 supplies sulfur; and frataxin (FXN) might provide the iron for initial ISC formation [14]. In addition to these molecules, mitochondrial chaperone protein HSPA9, which is required for erythropoiesis [15], is involved in the ISC formation [16] through the stabilization of frataxin, ISCU, and NFU [17] (Fig. 1c). Synthesized ISC (2Fe–2S) is provided to apoproteins, or used for 4Fe–4S cluster synthesis in mitochondria. Studies have confirmed that ABCB7 plays a crucial role in cytosolic ISC biogenesis by transporting unknown molecules from mitochondria to the cytosol [18]. Recently, a sulfur-containing compound or the glutathione-conjugated 2Fe–2S cluster has been proposed as a candidate molecule transported by ABCB7 [19].

During the maturation of erythroid cells, cytosolic aconitase plays an essential role in regulating iron transport and heme synthesis [20]. Cytosolic aconitase is a ISC protein that requires the 4Fe–4S cluster for its catalytic activity. Cytosolic aconitase also acts as an iron regulatory protein (IRP1), when it lacks the ISC [21]. Under iron-depleted conditions, IRP1 binds to the stem-loop structure of mRNA, known as the iron-responsive element (IRE), to suppress the translation of ferritin and erythroid-specific 5-aminolevulinate synthase (ALAS2, see Fig. 1c), or to induce the translation of TFR1 or DMT1 by protecting mRNA from nucleases [22]. IRP2 also binds to IRE under iron-depleted conditions and regulates translation similarly to IRP1, while IRP2 protein is poly-ubiquitinated and degraded by the proteasome under iron-rich conditions [23]. Therefore, a sufficient amount of iron and ISC, which inactivates IRPs, should be required for the appropriate translation of ALAS2. The IRE/IRP system plays a crucial role in the regulation of ALAS2 expression in erythroid cells [25], while it has been reported that TFR1 expression was regulated at the transcriptional level in erythroid cells [24].

Heme biosynthesis in erythroid cells

Since approximately 65% of the total body iron is found as a heme in hemoglobin [26], heme biosynthesis in erythroid cells should be the most important process for iron usage in the human body. Heme, which consists of protoporphyrin IX (PPIX) and ferrous iron, is a prosthetic group of several proteins, such as hemoglobin, myoglobin, cytochromes and catalases [27]. Heme biosynthesis is initiated in the mitochondrial matrix by 5-aminolevulinate synthase (ALAS), which condensates glycine and succinyl coenzyme A (CoA) to form 5-aminolevulinic acid (ALA). This is the rate-limiting step of heme biosynthesis in animals [28]. There are two isozymes for ALAS, namely, non-specific ALAS (ALAS-N or ALAS1) and erythroid-specific ALAS (ALAS-E or ALAS2). Each isozyme is encoded by a separate gene [28], and the transcriptional regulation of each gene is quite different [29, 30]. ALAS1 is ubiquitously expressed in all cells, including erythroblasts, whereas the expression of ALAS2 is restricted in erythroid cells. Heme production in erythroid progenitor cells is mainly catalyzed by ALAS2. During the proliferation and differentiation of embryonic [31] or adult [32] erythroid progenitor cells, transcription factor GATA-1 plays an essential role in the expression of erythroid-specific genes, such as globin genes. GATA-1 also regulates human ALAS2 gene via GATA-1 binding elements that were identified in the promoter region [33], as well as in erythroid-specific enhancer elements in the 8th intron [34] and the first intron [35].

ALA is exported to the cytosol across inner and outer mitochondrial membranes by unknown molecules, and then ALA is converted to coproporphyrinogen III (COPROgenIII) after four steps of sequential enzymatic reactions catalyzed by ALA dehydratase (ALAD, also known as porphobilinogen synthase), hydroxymethylbilane synthase (HMBS, also known as porphobilinogen deaminase), uroporphyrinogen III synthase (UROS) and uroporphyrinogen III decarboxylase (UROD). Erythroid-specific transcripts from each gene have been reported for ALAD [36], HMBS [37] and UROS [38], thereby enzymes from these transcripts may also contribute to erythroid heme synthesis. COPROgenIII is imported into mitochondria by ABCB6 [39] or SLC25A11 [40] and is oxidized by coproporphyrinogen oxidase (CPOX) to form protoporphyrinogen IX (PPgenIX) at the intermembrane space. PPgenIX is subsequently transported into the matrix by TMEM14C [41]. PPgenIX is subsequently converted to protoporphyrin IX (PPIX) by protoporphyrinogen oxidase (PPOX). As a last step of heme biosynthesis,

ferrochelatase (FECH) inserts ferrous iron into the center of PPIX to form heme. For details on the regulation of heme biosynthetic enzymes and porphyrin intermediates, please refer to Ref. [42]. Synthesized heme is exported to the cytosol by FLVCR1b [43] to form hemoglobin, and excess amount of heme in the cytosol is catabolized by heme oxygenases (HO-1 and HO-2) to biliverdin, ferrous iron and carbon dioxide [44], or simply exported by FLVCR1a across the plasma membrane [45].

Substrates of ALAS2 protein, glycine and succinyl CoA must be efficiently supplied in mitochondria for a large amount of heme synthesis in erythroid cells. It has been reported that SLC25A38 is highly expressed in erythroid cells [46], and act as a mitochondrial glycine importer [47]. Although succinyl CoA could be produced inside the mitochondria by α-ketoglutarate dehydrogenase complex or by succinyl CoA synthase, it has been reported that ATP-specific succinyl CoA synthase (A-SCS) forms a protein complex with ALAS2 protein but not ALAS1 protein via an ATP-specific β-subunit of SCS [48]. This protein complex formation might be important for the efficient supply of succinyl CoA to ALAS2.

Within these heme biosynthetic enzymes, the mutation of the ALAS2 gene is most frequently identified in patients with congenital sideroblastic anemia [49, 50]. Since human ALAS2 gene is mapped to X chromosome [30, 51], congenital sideroblastic anemia (CSA) caused by the mutation of ALAS2 gene exhibits X-linked inheritance pattern (X-linked sideroblastic anemia; XLSA). It has been reported that ring sideroblasts are observed in patients with erythropoietic protoporphyria (EPP) caused by a loss-of-function mutation of FECH gene. Although most EPP patients show mild microcytic, hypochromic anemia, the incidence of the ring sideroblasts feature in EPP patients is unknown [42, 52].

Congenital sideroblastic anemia (CSA)

As shown in Table 1, eleven genes are identified as responsible genes for CSA. Bergmann et al. identified mutations of ALAS2, SLC25A38, mitochondrial DNA and PUS1 in 37, 15, 2.5 and 2.5% of the CSA patients, respectively [50]. In Japan, mutations in ALAS2, SLC25A38 and mitochondrial DNA were identified in 67, 4, and 8% of the CSA patients, respectively [53]. Based on a clinical feature, CSA is subdivided into non-syndromic CSA or syndromic CSA. Non-syndromic CSA is characterized by anemia with ring sideroblast with/without secondary systemic iron overload.

SIDBA1; X-linked sideroblastic anemia (XLSA)

To date, a wide variety of missense mutations, nonsense mutations, and mutations at promoter or enhancer regions have been reported as a cause of XLSA [50, 52, 54, 55].

Pyridoxal 5′-phosphate (PLP) is an essential coenzyme of ALASs, and it has been reported that approximately half of patients with XLSA responded to the administration of a pharmacological dose of pyridoxine (pyridoxine-responsive XLSA) [52]. Thus, the ALAS2 gene mutation was first identified in a patient with pyridoxine-responsive sideroblastic anemia [56], and then in the pedigree of pyridoxine-responsive XLSA [57]. In Japanese pedigrees or isolated patients with pyridoxine-responsive anemia, a missense mutation in the ALAS2 gene was identified [58–60], and decreased

Table 1 List of causative genes for congenital sideroblastic anemia

Disease	Phenotype MIM number	Gene	Chromosome	Inheritance	Type of anemia
Non-syndromic					
XLSA (SIDBA1)	300751	ALAS2	Xp11.21	XR	Microcytic[a]
SIDBA2	205950	SLC25A38	3p22.1	AR	Microcytic
SIDBA3	616860	GLRX5	14q32.13	AR	Microcytic
SIDBA4	182170	HSPA9	5q31.2	AR	Microcytic
Syndromic					
XLSA/A	301310	ABCB7	Xq13.3	XR	Microcytic
PMPS	557000	Mitochondria	Mitochondria	Maternal/sporadic	Macrocytic
TRMA	249270	SLC19A2	1q24.2	AR	Macrocytic
MLASA1	600462	PUS1	12q24.33	AR	Macrocytic
MLASA2	613561	YARS2	12p11.21	AR	Macrocytic
SIFD	616084	TRNT1	3p26.2	AR	Microcytic
N/A	N/A	NDUFB11	Xp11.3	XR	Normocytic

SIDBA sideroblastic anemia, *XLSA/A* X-linked sideroblastic anemia with ataxia, *PMPS* Pearson's marrow pancreas syndrome, *TRMA* Thiamine-responsive megaloblastic anemia, *MLASA* myopathy, lactic acidosis, and sideroblastic anemia, *XR* X-linked recessive, *AR* autosomal recessive, *N/A* not available

[a]Macrocytic in part of female patients (see text)

enzymatic activity, as well as the responsiveness to the addition of PLP, of each mutant ALAS2 protein was confirmed in vitro.

Missense mutations in the ALAS2 gene were also identified in patients with pyridoxine-refractory XLSA, but the mutant enzymes showed normal enzymatic activity in vitro [61, 62]. However, it has been reported that some of these mutant proteins fail to associate with A-SCS [48, 62]. Since A-SCS could supply succinyl CoA to ALAS2 (see above), the failure of protein complex formation between ALAS2 and A-SCS resulted in decreased ALAS2 enzymatic activity in erythroblasts, causing sideroblastic anemia. Furthermore, the mutation or deletion of the ALAS2 promoter region has been reported as a cause of pyridoxine-refractory XLSA [55, 63]. Recently, novel erythroid-specific enhancer region of ALAS2 gene was identified in the 1st intron, which contains a single GATA-1 binding cis-element [35]. Several mutations or deletions involved in the GATA-binding site within this enhancer were also identified as causative mutations in 8 families of pyridoxine-refractory congenital sideroblastic anemia [35, 64]. Thus, the enhancer region at the 1st intron of ALAS2 gene should be examined in the pedigree of X-linked CSA, when no mutation is identified within coding region and exon–intron boundary region of the candidate genes.

Sideroblastic anemia in female patients with ALAS2 mutation

Although XLSA should show an X-linked inheritance pattern, several female patients with heterozygous ALAS2 mutation have been reported [55, 65–73], and approximately 25% of patients with pyridoxine-refractory anemia are female [74]. Two different mechanisms are proposed for female patients with heterozygous mutations of the ALAS2 gene. Cazzola et al. [67] hypothesized that acquired unbalanced lyonization was involved in the late-onset of the disease in female patients. A similar mechanism has been proposed as a cause of sideroblastic anemia in female patients with ALAS2 mutations by other groups [50, 65].

Recently, it has been reported that ALAS2 mutation is involved in pyridoxine-refractory macrocytic anemia in female patients. Sankaran et al. [72] studied a pedigree of macrocytic anemia, and found that the patients in this pedigree, who were heterozygous for a missense mutation in the ALAS2 gene, were all female. In vitro experiments using recombinant proteins showed that this mutation severely impairs the binding of cofactor to ALAS2 protein, resulting in the destabilization of the enzyme. Moreover, no transcript from the mutant ALAS2 allele was detected in peripheral reticulocyte of patients, although skewed X chromosome inactivation was not evident. In conjunction with an absence of male patients in the pedigree, the authors concluded that the mutation of the ALAS2 gene severely affected the stability of ALAS2 protein, thereby erythroid progenitor cells expressing the mutant allele became nonviable during terminal differentiation. Under such conditions, surviving erythroid progenitor cells expressing wild-type allele might differentiate under the pressure to produce a normal output of erythrocytes, resulting in macrocytosis [72]. Macrocytic anemia with heterozygous mutation of ALAS2 in female patients has been reported by several researchers [68, 71, 73], and these authors confirmed that the mutation identified in these patients caused a severe decrease in the enzymatic activity of ALAS2 proteins using bacterially-expressed recombinant protein [68, 73].

Iron metabolism in patients with XLSA

The presence of ring sideroblasts in bone marrow is a hallmark of sideroblastic anemia; however, the mechanism for the formation of iron deposits in mitochondria remains largely unknown. Energy-dispersive X-ray analysis revealed that ferric iron and phosphorus were accumulated in the mitochondrial matrix of sideroblasts, and the elemental component was distinct from that of ferritin aggregates or hemosiderin [75]. These authors speculated that a large amount of ferrous iron exceeding the capacity of heme synthesis was converted to a stable ferric state, and stored as ferric phosphate in the mitochondrial matrix. Then, Levi et al. [76] reported the cloning of mitochondrial ferritin (FTMT) gene, which encodes ferritin-heavy chain-like protein localized in mitochondria. This protein has ferroxidase activity and is highly expressed in erythroblasts isolated from patients with sideroblastic anemia but not in normal erythroblasts. Thus, authors speculated that FTMT expression might be induced by mitochondrial iron loading, and the excess iron is stored in FTMT as ferric iron to protect mitochondria from iron-induced oxidative stress. In fact, the protective roles of FTMT against oxidative stress have been reported [77, 78]. However, it is still unclear what stimulates FTMT expression, since FTMT mRNA does not have IRE for translational regulation. For appropriate hemoglobin production during erythroid differentiation, heme biosynthesis and mitochondrial iron incorporation should be co-ordinately regulated. ALAS2 expression is essential for heme synthesis in erythroid cells, and the function could not be compensated by ALAS1 [79]. For mitochondrial iron import in erythroid cells, mitoferrin-1 plays a crucial role, and the protein complex formation with ABCB10 is important for the stabilization of mitoferrin-1 protein. As discussed above, the erythroid transcription factor GATA-1 co-ordinately regulates the expression of ALAS2, ABCB10 and mitoferrin-1 at the transcriptional level. Moreover, Chung et al. [80] reported that GATA-1 induced the expression of mitochondrial outer membrane protein AKAP10, which could recruit protein

kinase A (PKA) to the outer membrane. Authors confirmed that FECH is a target of PKA for phosphorylation in vitro, as well as in cultured erythroid cells, and the phosphorylation of FECH by PKA increases the enzymatic activity of FECH. These results suggest that FECH is also indirectly activated by GATA-1 in erythroid progenitor cells. Taken together, heme biosynthesis (ALAS2 and FECH) and mitochondrial iron import complex (mitoferrin-1, ABCB10 and FECH) formation was, at least in part, co-ordinately regulated by the transcription factor GATA-1 during the maturation of erythroid cells. Under such conditions, decreased PPIX production by the ALAS2 mutation resulted in an increase of free iron in mitochondria, thereby forming iron deposits with FTMT to reduce oxidative stress mediated by free iron.

Systemic iron overload is also commonly observed in patients with XLSA, even in the blood transfusion-independent patients. The cause of the systemic iron overload is largely unknown; however, it has been suggested that three cytokines, which reduce hepcidin expression in the liver, are secreted from the erythroblasts of patients with this disease. In thalassemia, systemic iron overload is also frequently observed, and Tanno et al. found that the serum concentration of growth differentiation factor 15 (GDF15), a member of the transforming growth factor β superfamily, was increased in thalassemia patients compared with that in normal volunteers, and GDF15 could suppress hepcidin expression in the liver [81]. Subsequently, Ramirez et al. [82] reported that GDF15 is also increased in sera obtained from patients with acquired sideroblastic anemia. As a second and a third erythroid regulator, twisted gastrulation (TWSG1) [83] and erythroferron [84] were reported, respectively. However, no data are available for the TWSG1 or the erythroferron concentration in the sera of patients with congenital sideroblastic anemia.

SIDBA2; mutation of SLC25A38

As discussed above, SLC25A38 is a mitochondria glycine transporter, which is highly expressed in erythroid progenitor cells. During erythroid differentiation, erythroid progenitor cells need to synthesize a large amount of heme for hemoglobin production; therefore, the appropriate expression of the transporter for glycine, one of the substrates for ALAS2, should be essential. Thus, a loss-of-function mutation of the SLC25A38 gene should cause pyridoxine-refractory autosomal recessive congenital sideroblastic anemia [46]. Several missense, nonsense, and frame shift mutations of the SCL25A38 gene have been reported [46, 85–88].

SIDBA3; mutation of GLRX5

To date, the GLRX5 mutation has been identified in two families. Camaschella et al. reported a male sideroblastic anemia patient with a homozygous mutation in GLRX5 [89]. These authors confirmed that the mutation interfered the splicing of GLRX5 mRNA and diminished the GLRX5 function to produce ISC in mitochondria. Authors also confirmed the decreased aconitase activity in peripheral mononuclear cells isolated from a patient. Liu et al. reported a Chinese male patient who is a compound heterozygote of the missense mutation for GLRX5 gene, and observed the decreased aconitase activity in peripheral blood mononuclear cells [90]. As described above, decreased ISC formation converts aconitase to IRP1, resulting in the reduced translational efficiency of ALAS2 protein through IRE/IRP system (Fig. 1c). Moreover, decreased ISC assembly in mitochondrial matrix by GLRX5 deficiency may destabilize FECH protein, since FECH is required for the post-translational modification by ISC to maintain protein stability [91]. Thus, decreased enzymatic activity of ALAS2 due to insufficient translation, as well as reduced FECH activity by destabilization, both caused by the GLRX5 mutation, cause congenital sideroblastic anemia.

SIDBA4; mutation of HSPA9

Schmitz-Abe et al. [92] reported the mutation of HSP9, which encodes a mitochondrial HSP70 homologue, as a causative mutation of congenital sideroblastic anemia. In 6 pedigrees and 6 individuals, the authors identified various mutations, including frame shift mutations, in-frame deletion mutations, and missense and nonsense mutations. Furthermore, the authors found a single nucleotide polymorphism related to lower mRNA expression in majority of patients who have only one severely affected mutation in the other allele, resulting in a pseudodominant pattern of inheritance. The depletion of HSPA9 in erythroid cell lines inhibits erythroid differentiation, and it was suggested that this phenotype was caused by the translational inhibition of ALAS2 expression through the IRE/IRP system. Along with an insufficient supply of ISC to FECH protein, the loss-of-function mutation of HSPA9 suppressed the heme biosynthetic pathway solely in erythroid cells, thereby causing CSA.

Syndromic congenital sideroblastic anemia

Causative genes for syndromic CSA encode proteins expressed ubiquitously or in a limited number of tissues, although ALAS2, SLC25A38, GLRX5 or HSPA9 expression is limited in erythroid cells or highly induced in erythroid cells during their differentiation.

X-linked sideroblastic anemia with cerebellar ataxia

ABCB7 is involved in ISC biogenesis in the cytosol as discussed above, and missense mutations of the gene, resulting in partial loss-of-function, have been reported as responsible for X-linked sideroblastic anemia with cerebellar ataxia (XLSA/A) [93–96]. Similar to the cases of GLRX5 or HSPA9, ABCB7 deficiency decreases ISC biogenesis in the cytosol, thereby inducing the binding of IRP1 protein to IRE in the 5′ untranslated region of ALAS2 mRNA, which in turn may inhibit the translation of ALAS2 in erythroid cells [97].

Pearson's marrow pancreas syndrome (PMPS)

PMPS was first described by Pearson and characterized by the presence of refractory sideroblastic anemia and exocrine pancreatic insufficiency [98]. PMPS is caused by the deletion of mitochondrial DNA; however, the precise mechanism for sideroblastic anemia remains unknown, although it has been suggested that a defect of the respiratory chain in mitochondria due to the deletion of mitochondrial DNA might cause anemia [99].

Thiamine-responsive megaloblastic anemia associated with diabetes mellitus and deafness (TRMA)

In 1999, Labay et al. [100] identified a SLC19A2 mutation in patients with TRMA. Both SLC19A2 and SLC19A3 encode a high affinity thiamine transporter, while SLC19A2 is the sole transporter in marrow, pancreatic β-cells and a subset of cochlear cells [101, 102]. Since thiamine is required for transketolase in the non-oxidative branch of the pentose cycle, mutations in the transporter cause defects of de novo ribonucleotide production [103]. Moreover, α-ketoglutarate dehydrogenase in TCA cycle needs thiamine pyrophosphate as a cofactor to produce succinyl CoA, which is a substrate of ALAS [104]. Taken together, these findings may provide the pathologic background for megaloblastic sideroblasts in bone marrow, diabetes mellitus and deafness [105].

Myopathy, lactic acidosis, and sideroblastic anemia 1 (MLASA1)

Bykhovskaya et al. [106] reported the same missense mutation of the PUS1 gene, which encodes pseudouridine synthase 1 protein (PUS1p), in independent pedigrees with MLASA. There are three spliced variants, PUS1-1, -2, and -3 due to alternative splicing, and PUS1p derived from PUS1-1 has a mitochondria-targeting signal for mitochondrial translocation [107]. PUS1p catalyzes the modification of uridine to pseudouridine for mitochondrial transfer RNA (tRNA), and this process is essential for the proper function of tRNA, thus a loss-of-function mutation of PUS1 reduces the efficiency of translation in mitochondria, thereby causing a dysfunction of the respiratory chain for energy production. However, the pathological background for sideroblast formation is unknown.

Myopathy, lactic acidosis, and sideroblastic anemia 2 (MLASA2)

Riley et al. [108] identified the same missense mutation of YARS2 gene, which encodes mitochondrial tyrosyl-tRNA synthase, in two pedigrees with MLASA. These authors confirmed that this mutation reduced catalytic activity of complexes I, III, and IV of the respiratory chain, as well as the amounts of each component of the complex. Thus, the loss-of-function mutation of YARS2 gene reduced tyrosyl-tRNA in mitochondria, reducing translation of mitochondrial proteins involved in respiratory chain function. Several MLASA2 patients have been reported [109, 110], however, the origin of sideroblast formation remains unclear.

Sideroblastic anemia with immunodeficiency, fevers, and developmental delay (SFID)

Recently, Chakraborty et al. [111] reported the genetic mutation of TRNT1 gene, which encodes CCA-adding transfer RNA nucleotidyl transferase, as a cause of SFID. TRNT1 protein adds trinucleotides sequence (cytosine-cytosine-adenine, CCA) to the 3′ end of tRNA in the cytosol, as well as in mitochondria. This reaction is essential for aminoacylation of tRNA, thus, a partial defect of TRNT1 protein activity may account for the pathogenesis of the disease. Wedatilake et al. [112] analyzed the clinical course of their patients and previously reported cases and found that ring sideroblasts were reported in 13 of 18 patients, and was observed during

the investigation of unexpected febrile illness. However, the detailed mechanism for sideroblastic formation is unknown.

NDUFB11 deficiency

Quite recently, two different groups independently reported the deletion of three nucleotides in the NDUFB11 gene, which results in a phenylalanine deletion at 93rd amino acid residue (p.93delF) of the NDUFB11 protein, related to normocytic sideroblastic anemia and lactic acidosis [113, 114]. The NDUFB11 gene is mapped to the X chromosome (Xp11.23), and encodes a non-catalytic component of complex I of the mitochondrial respiratory chain and is essential for the assembly of an active complex I. As a disease related to the mutation of NDUFB11 gene, it was reported that the missense mutation of NDUFB11 causes microphthalmia with linear skin defects in female patients [115]. Moreover, another group described that novel missense mutation or frame shift mutation of the gene associated with histiocytoid cardiomyopathy [116], however, anemia was not documented in these patients. Thus, the reason why the p.93delF mutation is solely associated with sideroblastic anemia is unclear.

As discussed above, non-syndromic CSA is caused by a mutation of genes, of which expression is restricted in erythroid cells (e.g., ALAS2) or highly induced during terminal differentiation (e.g., GLRX5, HSPA9, and SLC25A38). In contrast, the expression of causative genes for syndromic CSA is not limited in erythroid cells, while the function of the gene products is related to iron–sulfur biogenesis (e.g., ABCB7), oxidative phosphorylation in mitochondria (e.g., PUS1, YARS2, TRNT1, and NDUFB11), or uptake of thiamine (SLC19A2). However, the precise mechanism for sideroblast formation remains unknown. While increased expression of FTMT in sideroblasts might be a key event for mitochondrial iron accumulation, it is still unclear whether iron accumulation induces FTMT expression or the other stimuli induce its expression. Thus, the establishment of model cells or model animals, which produce ring sideroblasts, awaits future analysis.

Compliance with ethical standards

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

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