Iron & Disease - Section 2

Congenital sideroblastic anemias: From molecular causes to treatment

Sarah Ducamp, Mark D. Fleming

Department of Pathology, Boston Children’s Hospital and Harvard Medical School, Boston, USA

Introduction

Abnormal erythroblast mitochondrial iron deposits are the hallmark of the sideroblastic anemias (SAs). On Prussian blue stained bone marrow aspirate smears, the iron-overloaded mitochondria surround the erythroblast nucleus, giving rise to the name “ringed sideroblast” (RS). There are congenital and acquired forms of SA. Acquired RS can result from environmental exposures such as alcohol, certain drugs (e.g., isoniazid, linezolid, or chloramphenicol) or nutritional deficiencies (e.g., copper). They may also be seen in acquired myelodysplastic syndromes, in which case they are often associated with clonal somatic mutations in proteins/genes involved in mRNA splicing. This review will focus on recent advances in understanding the congenital SAs (CSAs), which are largely monogenic diseases resulting from inherited or de novo germline mutations in proteins/genes associated with heme biosynthesis, mitochondrial iron-sulfur cluster (ISC) biogenesis, or mitochondrial protein synthesis and/or respiration (Figure 1). These pathways are highly interconnected. For example, heme and ISC are required for multiple oxidative phosphorylation (OXPHOS) complexes, some of which are encoded by the mitochondrial genome. A general overview of the diagnosis and management of CSA was published in 2014, at which time there were 8 genetically defined causes of CSA (Figure 1). In the last 4 years, 5 novel CSA genes have been discovered: ATP6, TRNT1, HSPA9, LARS2, and NDUFB11. In addition, regulatory mutations in ALAS2 have been described in patients with X-linked sideroblastic anemia (XLSA). Finally, the association of RS with occasional patients with β-thalassemia and hemoglobinopathies have been described.

Advances in CSA due to heme synthesis defects

XLSA is the most common form of CSA, and is due to mutations in ALAS2, the erythroid-specific isoform of the heme biosynthetic enzyme aminolevulinate acid synthase. Whereas missense mutations that result in altered catalytic properties or enzyme stability are the rule for this disease, recently mutations in a GATA1 binding site in a transcriptional enhancer in intron 1 of the ALAS2 gene have been described in multiple families. Unlike all other mutations found in males with the disease, these mutations lead to decreased expression of the mRNA and very reduced amounts of a structurally normal protein. As such, it highlights the probability that mutations in tissue-specific gene expression will underlie a subset of CSA and other inherited RBC disorder phenotypes. Until recently, there has been very limited success in establishing cellular and animal models of the CSAs. For example, Alas2 null and GATA1-enhancer binding site mutant mice die in utero. Importantly, mice do not develop ringed sideroblasts, but instead develop siderocytes - enucleated red blood cells containing iron granules. Hatta and colleagues, however, very recently demonstrated that supplementation of induced pluripotent stem cell (iPS)-derived erythroblasts with exogeneous non-transferrin bound iron was critical for the generation of RS in vitro. This strategy was also adapted to CRISPR-Cas9 edited HIDEP cells, lacking a GATA1 binding domain in the ALAS2 first intron. Thus, for the first time, there is a relevant in vitro model of RS formation that can be employed to test potential therapeutics and further explore the pathogenesis. Mutations in the mitochondrial solute carrier SLC25A38, thought to be a high affinity transporter required for mitochondrial glycine import, were previously identified in patients with non-syndromic CSA. Work in yeast and in zebrafish knockdown

Take Home Messages

- Ringed sideroblasts are the hallmark of diseases called Sideroblastic Anemias, which can be either acquired or congenital. Congenital sideroblastic anemias result from defects in heme biosynthesis, mitochondrial iron-sulfur cluster (ISC) biogenesis, or mitochondrial protein synthesis and/or respiration.
- Five new congenital sideroblastic anemia genes (ATP6, TRNT1, HSPA9, LARS2 and NDUFB11) as well as mutations in regulatory element of ALAS2 have been identified this 4 last years. Mutations in 12 genes (8 autosomal, 3 X-linked, and one mitochondrial) and multigenic mitochondrial DNA deletions are responsible for CSA; the genetic defect is still unknown for approximately one-third of patients.
- Recent development of in vitro models of the CSAs offers the potential for developing a deeper understanding of the pathogenesis and treatment of these uncommon diseases.
models supported the importance of altered glycine metabolism to the pathogenesis of the disorder, and also revealed a potential dependence of the phenotype on altered folate metabolism. Supplementation of glycine in combination with reduced folate was able to ameliorate the zebrafish model anemia, but, unfortunately, a limited evaluation of this combination therapy in SLC25A38 CSA patients failed to demonstrate any benefit.

**HSPA9 and erythroid ISC biosynthesis**

Altered ISC assembly has been implicated in the pathogenesis of CSA due to mutations in GLRX5 and ABCB7. HSPA9, is a mitochondrial HSP70 chaperone involved in ISC assembly and in erythroid differentiation. A number of non-syndromic CSA patients have been identified with apparently recessively or dominantly inherited disease due to HSPA9 mutations. Patients with recessively inherited phenotype often have more severe disease due to compound heterozygous effects of a severe loss-of-function mutation and a presumptively milder missense variant, whereas patients with apparently dominant disease often have a milder phenotype associated with a deleterious HSPA9 mutation in trans of a common variant (rs10117T) predicted to be an exonic splice enhancer that reduces HSPA9 mRNA expression.

**Recurrent NDUFB11 mutation in CSA**

A recurrent hemizygous mutation, c.276_278del, p.F96del, in NDUFB11, a structural subunit of OXPHOS complex I, was identified in multiple unrelated patients by whole exome sequencing. These patients often have subtle syndromic features including lactic acidosis, myopathy and/or mild cognitive disability. Decreased NDUFB11 expression and complex I activity were confirmed in patient-derived fibroblasts. CRISPR-Cas9 edited K562 erythroleukemia cells demonstrated a proliferation defect without effect on hemoglobinization. NDUFB11 null variants result in either histiocytoid cardiomyopathy or microphthalmia with linear skin defects in carrier female and prenatal lethality in males, indicating that the NDUFB11F96del phenotype may be an allele-specific disorder.

**New genes responsible for mitochondrial myopathy with lactic acidosis and sideroblastic anemia**

Mitochondrial myopathy, lactic acidosis and sideroblastic anemia (MLASA) can result from mutations in PUS1 and YARS2, which encode an enzyme involved in global tRNA maturation and the mitochondrial tyrosyl-tRNA synthetase, respectively. Deficiency of either protein is thought to result in decreased efficiency of mitochondrial translation and OXPHOS complex deficiency, in a manner that may be comparable to alterations due to mitochondrial DNA deletions (that commonly remove mitochondrial tRNA genes) in the prototypical syndromic CSA, Pearson Marrow-Pancreas Syndrome (PMPS). A recurrent point mutation in ATP6, a mitochondrially encoded subunit of respiratory complex V (ATP synthase), has been discovered in one patient with a phenotype described as “MLASA-plus” and several patients with a less-severe neurometabolic syndrome similar to MLASA-plus has been described in one patient with compound heterozygous mutations in the mitochondrial leucyl-tRNA synthetase, LARS2.

**TRNT1 and sideroblastic anemia, immunodeficiency, periodic fevers and developmental delay**

The template-independent RNA polymerase TRNT1 catalyzes the addition of a cytosine-cytosine-adenine (CCA) trinucleotide to all cytosolic and mitochondrial tRNAs. Recessive, incomplete TRNT1 loss-of-function results in a syndromic form of CSA associated with B-cell immunodeficiency, periodic fevers, and developmental delay (SIFD). Since the original description of SIFD, the spectrum of TRNT1 mutation association pheno-
types, has broadened substantially, and now includes patients with predominantly or solely neurologic (retinitis pigmentosa), hematologic (CSA), or immunodeficiency phenotypes. Given the involvement of TRNT1 in both cytosolic and mitochondrial translation, it is not surprising that the phenotype is highly variably severe and pleiotropic. Unlike most other forms of syndromic CSA, patients with SIFD characteristically have very low MCVs, possibly due to a likely unique, severe effect on globin translation in the RBC. Recent work has shown that there is indeed an OXPHOS defect in patient-derived fibroblasts,\(^1\) \(^7\) which could be secondary to alterations in any of the CSA disease associated pathways. Interestingly, incompletely processed tRNAs accumulate in patient cells, which may induce an innate immune response, eventually in the “aseptic sepsis” episodes characteristic of the complete syndromic phenotype, and has suggested that these crises may be effectively managed with tumor necrosis factor (TNF) pathway inhibitors.\(^1\)^\(^8\)

**Future perspectives**

In the context of recent advances and with the advent of next generation sequencing, we are now able to identify the genetic cause of CSA in at least two-thirds of cases (unpublished) allowing for the precise classification of disease as well as enabling the rational design of therapeutics tailored to each genotype. To this point, the only specific therapies for the CSAs are pyridoxine or thiamine supplementation, which benefits approximately one-half of XLSA patients and two-third of thiamine-responsive megaloblastic anemia (TRMA) patients, respectively; TRMA being due to mutations in SLC19A2 a thiamine transporter. Development of new animal and in vitro models for the CSAs is a critical next step that will contribute significantly to our understanding of the pathogenesis and ultimately therapies for these diseases.

**References**

1. Bottomley SS, Fleming MD. Sideroblastic anemia: diagnosis and management. Hematol Oncol Clin North Am 2014;28:653-70.

2. Burrage LG, Tang S, Wang J, et al. Mitochondrial myopathy, lactic acidosis, and sideroblastic anemia (MLASA) plus associated with a novel de novo mutation (m.8969G>A) in the mitochondrial encoded ATP6 gene. Mol Genet Metab 2014;113:207-12.

3. A recurrent mutation in mitochondrial encoded ATP6, a subunit of ATP synthase, is present in a heteroplasmic state in patients some with a severe MLASA-like disorder.

4. Chakraborty PK, Schmitz-Abe K, Kennedy EK, et al. Mutations in TRNT1 cause congenital sideroblastic anemia with immunodeficiency, fevers, and developmental delay (SIFD). Blood 2014;124:2867-71.

5. A new syndromic sideroblastic anemia phenotype, SIFD, is due to recessive mutations in TRNT1, which catalyzes the addition of a cytosine-cytosine-adenine (CCA) trinucleotide to all cytosolic and mitochondrial tRNAs.

6. Schmitz-Abe K, Ciesielski SJ, Schmidt PJ, et al. Congenital sideroblastic anemia due to mutations in the mitochondrial HSPA9. Blood 2015;126:2734-8.

7. Mutations in HSPA9, a crucial chaperone of mitochondrial iron sulfur assembly are responsible for non-syndromic CSA, highlighting the importance of this process in the CSA pathogenesis.

8. Riley LG, Rudinger-Thirion J, Schmitz-Abe K, et al. LARS2 variants associated with hydrops, lactic acidosis, sideroblastic anemia, and multisystem failure. JIMD Rep 2016;28:49-57.

9. Lichtenstein DA, Crispin AW, Sendamarai AK, et al. A recurring mutation in the respiratory complex I protein NDUFB11 is responsible for a novel form of X-linked sideroblastic anemia. Blood 2016;128:1913-7.

10. A recurrent mutation in NDUFB11, an X-linked gene that encodes a subunit of respiratory complex I and associated with defective mitochondrial energy are responsible for a mildly syndromic form of CSA.

11. Torraco A, Bianchi M, Verrigni D, et al. A novel mutation in NDUFB11 unvels a new clinical phenotype associated with lactic acidosis and sideroblastic anemia. Clin Genet 2017;91:441-7.

12. Campagna DR, de Bie CJ, Schmitz-Abe K, et al. X-linked sideroblastic anemia due to ALAS2 intron 1 enhancer element GATA-binding site mutations. Am J Hematol 2014;89:315-9.

13. Kaneko K, Furuyama K, Fujiwara T, 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 2014;99:252-61.

14. Cattivelli K, Campagna DR, Schmitz-Abe K, et al. Ringer sideroblasts in beta-thalassemia. Pediatr Blood Cancer 2017;64.

15. Hatta S, Fujiwara T, Yamamoto T, et al. A defined culture method enabling the establishment of ring sideroblasts from induced pluripotent cells of X-linked sideroblastic anemia. Haematologica 2018;103:e188-e191.

16. Addition of non-transferrin bound iron to media appears to be essential to in vitro ringed sideroblast formation in erythroblasts derived from XLSA patients.

17. Saito K, Fujiwara T, Hatta S, et al. Establishment and characterization of in vitro model of X-linked sideroblastic anemia. Blood 2017;130:171.

18. Fernandez-Murray JP, Prykhozhij SV, Dufay JN, et al. Glycine and Folate ameliorate models of congenital sideroblastic anemia. PLoS Genet 2016;12:e1005783.

19. Campagna DR, de Bie CJ, Schmitz-Abe K, et al. Study of glycine and folic acid supplementation to ameliorate transfusion dependence in congenital sideroblastic anemia. Blood 2017;130:171.

20. Saito K, Fujiwara T, Hatta S, et al. Establishment and characterization of in vitro model of X-linked sideroblastic anemia. Blood 2017;130:171.

21. Fernandez-Murray JP, Prykhozhij SV, Dufay JN, et al. Glycine and Folate ameliorate models of congenital sideroblastic anemia. PLoS Genet 2016;12:e1005783.