scientific reports

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Congenital sideroblastic anemia model due to *ALAS2* mutation is susceptible to ferroptosis

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X-linked sideroblastic anemia (XLSA), the most common form of congenital sideroblastic anemia, is caused by a germline mutation in the erythroid-specific 5-aminolevulinate synthase (*ALAS2*) gene. In XLSA, defective heme biosynthesis leads to ring sideroblast formation because of excess mitochondrial iron accumulation. In this study, we introduced *ALAS2* missense mutations on human umbilical cord blood-derived erythroblasts; hereafter, we refer to them as XLSA clones. XLSA clones that differentiated into mature erythroblasts showed an increased frequency of ring sideroblast formation with impaired hemoglobin biosynthesis. The expression profiling revealed significant enrichment of genes involved in ferroptosis, which is a form of regulated cell death induced by iron accumulation and lipid peroxidation. Notably, treatment with erastin, a ferroptosis inducer, caused a higher proportion of cell death in XLSA clones. XLSA clones exhibited significantly higher levels of intracellular lipid peroxides and enhanced expression of BACH1, a regulator of iron metabolism and potential accelerator of ferroptosis. In XLSA clones, BACH1 repressed genes involved in iron metabolism and glutathione synthesis. Collectively, defective heme biosynthesis in XLSA clones could confer enhanced BACH1 expression, leading to increased susceptibility to ferroptosis. The results of our study provide important information for the development of novel therapeutic targets for XLSA.

Sideroblastic anemia is composed of a group of congenital and acquired disorders that share the characteristic presence of bone marrow ring sideroblasts, which represent excess mitochondrial deposition of iron[1,2]. In adults, these syndromes are often found to be related to myelodysplastic syndrome (MDS), which results from a mutation in the RNA-splicing machinery component splicing factor 3b, subunit 1 (*SF3B1*) gene[3]. On the other hand, the congenital forms of sideroblastic anemia (congenital sideroblastic anemia: CSA) are rare and constitute a diverse class of inherited disorders[1,2]. CSA has been considered to be caused by a mutation in the genes involved in heme biosynthesis, iron-sulfur cluster biosynthesis, and mitochondrial protein synthesis[2,4].

X-linked sideroblastic anemia (XLSA) is the most common form of CSA and is caused by germline mutations in the erythroid-specific 5-aminolevulinate synthase (*ALAS2*) gene, which encodes the first and rate-limiting enzyme of heme biosynthesis[1,4]. The enzyme converts glycine and succinyl-coenzyme A to 5-aminolevulinic acid (ALA) and requires pyridoxal 5′-phosphate (vitamin B6) as a cofactor[1,2,4]. Clinically, patients with XLSA are predominantly hemizygous males who present with the disease most commonly < 40 years and exhibit hypochromic microcytic anemia with a varying degree that is accompanied by systemic iron overload[1,2,4,5]. Heterozygous female carriers can also develop sideroblastic anemia caused by skewed X-chromosome inactivation, which is related to aging[6–12]. Most XLSA-associated mutations are missense substitutions that result in a loss of protein functionality, whereas mutations in the *ALAS2* regulatory region have also been reported[2,4,13,14]. As different *ALAS2* missense mutations show different effects on the protein's function, XLSA patients demonstrate varying degrees of disease severity[15]. Even female patients with macrocytic sideroblastic anemia due to heterozygous *ALAS2* mutations have been reported, which is attributed to the severe loss-of-function mutation in *ALAS2*[16,17].

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