REGULAR ARTICLE

blood advances

Azacitidine is a potential therapeutic drug for pyridoxine-refractory female X-linked sideroblastic anemia

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Key Points

- A patient-derived iPSC model recapitulates defective erythroid maturation in female XLSA.
- Azacitidine reactivates the silent wild-type *ALAS2* allele and ameliorates inefficient erythropoiesis in iPSC-derived HPCs from female XLSA.

X-linked sideroblastic anemia (XLSA) is associated with mutations in the erythroid-specific δ-aminolevulinic acid synthase (*ALAS2*) gene. Treatment of XLSA is mainly supportive, except in patients who are pyridoxine responsive. Female XLSA often represents a late onset of severe anemia, mostly related to the acquired skewing of X chromosome inactivation. In this study, we successfully generated active wild-type and mutant *ALAS2*-induced pluripotent stem cell (iPSC) lines from the peripheral blood cells of an affected mother and 2 daughters in a family with pyridoxine-resistant XLSA related to a heterozygous *ALAS2* missense mutation (R227C). The erythroid differentiation potential was severely impaired in active mutant iPSC lines compared with that in active wild-type iPSC lines. Most of the active mutant iPSC-derived erythroblasts revealed an immature morphological phenotype, and some showed dysplasia and perinuclear iron deposits. In addition, globin and *HO-1* expression and heme biosynthesis in active mutant erythroblasts were severely impaired compared with that in active wild-type erythroblasts. Furthermore, genes associated with erythroblast maturation and karyopyknosis showed significantly reduced expression in active mutant erythroblasts, recapitulating the maturation defects. Notably, the erythroid differentiation ability and hemoglobin expression of active mutant iPSC-derived hematopoietic progenitor cells (HPCs) were improved by the administration of δ-aminolevulinic acid, verifying the suitability of the cells for drug testing. Administration of a DNA demethylating agent, azacitidine, reactivated the silent, wild-type *ALAS2* allele in active mutant HPCs and ameliorated the erythroid differentiation defects, suggesting that azacitidine is a potential novel therapeutic drug for female XLSA. Our patient-specific iPSC platform provides novel biological and therapeutic insights for XLSA.

Introduction

Congenital sideroblastic anemias are a group of heterogeneous inherited diseases characterized by impaired heme synthesis and mitochondrial iron overload with ring sideroblasts in the bone marrow. X-linked sideroblastic anemia (XLSA) is the most common hereditary sideroblastic anemia and is caused

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RNA sequencing (RNA-seq) data are available at Gene Expression Omnibus (GEO; accession number GSE176008).

For the original data, please e-mail the corresponding authors.

The full-text version of this article contains a data supplement.