SURF1 knockout pig: a powerful model to implement In Utero Fetal Gene Therapy for Leigh Syndrome

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Objective

Mutations in the SURF1 gene represent one of the major causes of Leigh Syndrome a fatal mitochondrial necrotizing encephalopathy characterized by psychomotor regression and lactic acidosis with a peak of mortality before the age of three. LS affects 1 in 36.000 new-borns and represent the most frequent paediatric manifestation of mitochondrial disease. SURF1 is an inner mitochondrial membrane protein involved in the cytochrome C oxidase (COX) assembly, the fourth complex of the mitochondrial oxidative phosphorylation (OXPHOS) system. The loss of SURF1 reduces the formation of the fully assembled COX, with subsequent impairment of mitochondrial energy production. The molecular mechanisms underlying the neuronal pathology in LS due to SURF1 mutations are still not fully elucidated in vivo, given the lack of adequate model systems that has also limited the therapeutic development. Several attempts to generate SURF1-deficient LS animal models have been made in the last decade, with controversial results. SURF1 knockout mice failed to recapitulate the neurological phenotypes seen in humans and even led to a prolonged lifespan, despite mild COX deficiency. Pigs are an attractive alternative model for human diseases because of their metabolic, physiological, and genetic similarity to humans. Objectives: 1) to generate a Surf1KO pig model of LS; 2) to restore SURF1 function through an In Utero Fetal Gene Therapy (IUFGT) approach based on ultrasound-guided injection of scAAV2/9-Surf1WT in the umbilical vein of Surf1KO pig fetuses.

Methods

Animal model generation: We determined the complete sequence of the swine SURF1 gene and disrupted it in pig primary fibroblast cell lines using both TALENs and CRISPR/Cas9 genome editing systems. Resulting Surf1 knockout fibroblasts were then used to generate SURF1KO pigs by Somatic Cell Nuclear Transfer (SCNT). IUFGT set-up: In the first instance, recombinant AAV2/9 vector packaging self-complementary GFP under the CAG promoter will be injected at different doses (1011,1012, 1013 vg/Kg) to pig fetuses (E70, gestational duration 115 days) via intrauterine ultrasound-guided injection through the umbilical cord vessels. Fetal growth will be monitored by ultrasound until birth. Stillborn and new-born piglets will be analysed by PCR and immunofluorescence in different tissues to determine the GFP reporter expression, establish the optimal viral concentration, and investigate its distribution in brain and peripheral tissues. Subsequently, the same procedure will be repeated using the scAAV2/9 expressing human Surf1 cDNA at the optimal concentration established in the preliminary experiments.

Results

The clinical phenotype associated with the ablation of SURF1 in the pig, was highly severe, resembling that of LS patients, and characterized by failure to thrive, persistent tremors, muscle weakness, markedly reduced growth and elevated perinatal mortality compared to control littermates. SURF1KO piglets showed reduced complex IV activity in muscle and jejunum. Brain analysis revealed a significant reduction of the cortical thickness of the cerebral cortex grey matter at early postnatal ages and a disorganized cortical structure with several immature neurons resulting from Doublecortin (DCX) staining and increased gliosis suggested by Iba1 staining.

Conclusion

The early onset of a severe clinical phenotype and the presence of several immature neurons in the brain of SURF1KO newborn piglets, suggest that SURF1 defect could impair the neural precursor cellular metabolism in the early phase of the development leading to the onset of the neurological phenotypes. So, IUFGT may represent a valid therapy for LS offering the possibility of early intervention before the occurrence of irreversible damage, in a temporal window in which undamaged neurons can be harnessed for transgenic protein production, increasing the chances to restore OXPHOS activity and complete neural differentiation.