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Modeling of Endothelial Cell Dysfunction Using Patient-Derived Induced Pluripotent Stem Cells

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The objective of this study was to investigate the efficacy of CRISPR/Cas9-mediated A4GALT suppression in rescuing endothelial dysfunction in Fabry disease (FD) endothelial cells (FD-ECs) derived from human induced pluripotent stem cells (hiPSCs). We differentiated hiPSCs (WT (wild-type), WTC-11), GLA-mutant hiPSCs (GLA-KO, CMC-Fb-002), and CRISPR/Cas9-mediated A4GALT-KO hiPSCs (GLA/A4GALT-KO, Fb-002-A4GALT-KO) into ECs and compared FD phenotypes and endothelial dysfunction. We also analyzed the effect of A4GALT suppression on reactive oxygen species (ROS) formation and transcriptome profiles through RNA sequencing. GLA-mutant hiPSC-ECs (GLA-KO and CMC-Fb-002) showed downregulated expression of EC markers and significantly reduced α-GalA expression with increased Gb-3 deposition and intra-lysosomal inclusion bodies. However, CRISPR/Cas9-mediated A4GALT suppression in GLA/A4GALT-KO and Fb-002-A4GALT-KO hiPSC-ECs increased expression levels of EC markers and rescued these FD phenotypes. GLA-mutant hiPSC-ECs failed to form tube-like structure in tube formation assays with, showing significantly decreased migration of cells into the scratched wound area. In contrast, A4GALT suppression improved tube formation and cell migration capacity. Western blot analysis revealed that MAPK and AKT phosphorylation levels were downregulated whereas SOD and catalase were upregulated in GLA-KO hiPSC-ECs. However, suppression of A4GALT restored these protein alterations. RNA sequencing analysis demonstrated significant transcriptome changes in GLA-mutant EC, especially in angiogenesis, cell death, and cellular response to oxidative stress. However, these were effectively restored in GLA/A4GALT-KO hiPSC-ECs. CRISPR/Cas9-mediated A4GALT suppression rescued FD phenotype and endothelial dysfunction in GLA-mutant hiPSC-ECs, presenting a potential therapeutic approach for FD-vasculopathy.

Keywords: Fabry disease, hiPSCs, CRISPR/Cas9, endothelial cells, endothelial dysfunction