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Delivery of A4GALT targeting siRNA-lipid nanoparticle rescues fabry disease phenotypes in hiPSC derived endothelial cell and podocyte models

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Objectives : We investigated the therapeutic efficacy of a novel polyhistidine-incorporated lipid nanoparticle (pHis/LNP) grafted with A4GALT siRNA in Fabry disease (FD) endothelial cells (FD-ECs) and podocytes (FD-PCs) derived from human induced pluripotent stem cells (hiPSCs).
Methods : First, we developed a novel polyhistidine (pHis)-incorporated lipid nanoparticle (pHis/LNP) for the delivery of therapeutic globotriaosylceramide (Gb3) synthase siRNAs using a microfluidic device with pHis as a biocompatible method of endosome escape. Second, we differentiated GLA-mutant (kock-out) hiPSCs (GLA-KO) into ECs or PCs and then treated with A4GALT siRNA-pHis/LNP for 24 hours. Third, we comparatively analyzed the FD phenotype, such as alpha-galactosidase-A enzyme (α-GalA) activity, globotriaosylceramide (Gb-3) deposition, and zebra body formation under electron microscopy (EM) in each cell population. Finally, we examined the changes of transcriptome levels using RNA-sequencing.
Results : The presence of LNP in the cells was confirmed through fluorescence light microscopy (LM) and EM after 24 hours of pHis/LNP treatment in GLA-KO hiPSC-ECs and GLA-KO hiPSC-PCs. The stability of cell viability was also verified after pHis/LNP treatment by concentration. Western blot analysis shows the decreased GLA protein expression in the GLA-mutants and A4GALT siRNA-pHis/LNP treated populations compared to the WTC11, and the decrease of A4GALT expression was identified only in the A4GALT siRNA-pHis/LNP treated population. In GLA-mutants, we observed the decreased α -GalA expression, the increased Gb-3 deposition, and intra-lysosomal inclusion bodies such as zebra body. These FD phenotypes were attenuated after A4GALT siRNA-pHis/LNP treatment in GLA-mutants. RNA-sequencing analysis demonstrated significant transcriptome changes in GLA-mutants. However, these were restored in A4GALT siRNA-pHis/LNP treated population.
Conclusions : Suppression of A4GALT using siRNA-pHis/LNP treatment could rescue FD phenotype in FD-ECs and FD-PCs. Our data suggested that siRNA-pHis/LNP can be proposed as a new therapeutics for the treatment of FD.