Abstract

Purpose. The purpose of this study is to establish and analyze a cell model of Leber congenital amaurosis type 16 (LCA16), which is caused by mutations in the *KCNJ13* gene encoding Kir7.1, an inward rectifying potassium ion channel.

Methods. The two gRNAs specific to the target sites in the *KCNJ13* gene were designed and *KCNJ13* knockout (KO) human-induced pluripotent stem cells (hiPSC) were generated using the CRISPR/Cas9 system. The *KCNJ13* KO hiPSCs were differentiated into retinal pigment epithelial cells (hiPSC-RPE). The *KCNJ13* KO in hiPSC-RPE was confirmed by immunostaining. Phagocytic activity of hiPSC-RPE was assessed using uptake of fluorescently labeled porcine photoreceptor outer segments (POS). Phagocytosis-related genes in RPE cells were assessed by quantitative polymerase chain reaction (PCR).

Results. Most of the translated region of the *KCNJ13* gene was deleted in the *KCNJ13* KO hiPSCs by the CRISPR/Cas9 system and this confirmed that the Kir7.1 protein was not present in RPE cells induced from hiPSCs. Expression of RPE marker genes such as *BEST1* and *CRALBP* was retained in the wild type (WT) and in the *KCNJ13*-KO hiPSC-RPE cells. However, phagocytic activity and expression of phagocytosis-related genes in the *KCNJ13*-null hiPSC-RPE cells were significantly reduced compared to those of WT.

Conclusions. We succeeded in generating an RPE model of LCA16 using hiPSCs. We suggest that Kir7.1, an inward rectifying potassium ion channel, is required for phagocytosis of POS by RPE cells and that impaired phagocytosis in the absence of Kir7.1 would be involved in the retinal degeneration found in LCA16.

Keywords: Kir7.1, *KCNJ13*, human-induced pluripotent cells, retinal pigment epithelium, phagocytosis