

Poster

'Ex vivo' gene correction of PRPF31 c.165G>A mutation causing retinitis pigmentosa

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ABSTRACT

Motivation: Retinitis pigmentosa (RP) is the most common form of retinal dystrophy, a group of blinding diseases characterized by progressive photoreceptor death, with a prevalence of 1 in 4000. RP is highly-heterogeneous, with 15% of autosomal dominant cases caused by mutations in the pre-mRNA processing factors (PRPFs), components of the spliceosome.

To date, there are no effective treatments for RP. Gene editing is a rapidly evolving field that may in the future, allow the repair of a mutated endogenous locus. CRISPR/Cas9 system has a mechanism of action based on nucleotide recognition of target DNA by engineered single-guide RNA (sgRNA) and Cas9 endonuclease activity. Genomic edition of patient-derived induced pluripotent stem cells (iPSCs) would allow autologous transplantation of repaired cells, once differentiated to retinal cell types.

Methods: iPSCs obtained from a RP patient with a PRPF31 c.165G>A mutation were the starting biological material. Pluripotency of the iPSCs was checked by immunofluorescence (IF) analysis.

Disease phenotyping of the cell line was performed by IF for PRPF31 and for the ciliary protein ARL13B, as PRPF31 mutations have been previously described to affect cilia.

sgRNAs directed to the mutation were designed using the web crispor.tefor.net. The best sgRNA and a ssODN template, covering the mutation site, were synthesized by IDT. The sgRNA-CRISPR/Cas9 complex was assembled and co-transfected with the ssODN into the iPSCs. FACs was used to measure the efficiency and to select transfected cells. A bulk transfected cell population was analyzed by Sanger sequencing to check for HR-mediated knock-in. Selection of individual iPSC clones and genotyping is being performed to search for corrected clones.

Results: Positive labeling for OCT4, NANOG, SSEA3, SSEA4 and TRA-1-81 showed pluripotency of the iPSC line. PRPF31 immunolocalization and quantification have been used to phenotype the iPSC line compared to a healthy control. Sanger sequencing of the genomic DNA showed successful editing of the mutation in the bulk population of transfected cells. Different culture conditions were tested for iPSC clonal selection. Best conditions provided a 0.8 % of efficiency. The CRISPR/Cas9-corrected iPSC clone will be differentiated to retinal pigment epithelium (RPE) and photoreceptors, in parallel with uncorrected PRPF31-iPSCs, to establish if in situ gene editing restores key cellular and functional phenotypes associated with this type of RP

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