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Generation of iPSC-Derived RGCs for Modeling Dominant Optic Atrophy †

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Abstract: Dominant optic atrophy (DOA), mainly caused by pathogenic variants in *OPA1*, is one of the most frequent forms of hereditary optic neuropathy. *OPA1* is involved in mitochondrial dynamics or oxidative phosphorilation, among other functions. Hence, mutations in this gene cause the degeneration of retinal ganglion cells (RGCs) leading to reduced visual acuity. In this work, we have used induced pluripotent stem cell (iPSC) technology to generate RGCs, starting from an iPSC line created from fibroblasts from a DOA patient and also its CRISPR isogenic control. The generated RGCs showed expression of BRN3A, SNCG or THY1, and potentially would serve as a platform for DOA modeling.

Keywords: dominant optic atrophy; DOA; optic neuropathy; OPA1; induced pluripotent stem cells; iPSCs; mitochondria; retinal ganglion cells; CRISPR/Cas9; disease model

1. Introduction

Dominant optic atrophy or DOA is a rare progressive disease and one of the most frequent forms of hereditary optic neuropathy [1]. This condition is mainly triggered by pathogenic variants in the nuclear gene *OPA1*, which encodes a dynamic-related protein localized in the mitochondrial inner membrane [2-3]. Among their functions, *OPA1* plays a key role in mitochondrial dynamics, cell survival, oxidative phosphorilation or the maintenance of mitochondrial DNA (mtDNA). Mutations in this gene cause a decrease of energy production capacity leading to the degeneration of retinal ganglion cells (RGCs) and their axons [4]. This implies optic nerve atrophy and reduced visual acuity, coming with legal blindness in many cases. DOA can also be syndromic with extra-ocular features like ataxia, myopathy, chronic ophtalmoplegia and sensorineural deafness, condition known as DOA 'plus' [5]. Currently, there is no effective treatment for DOA due in part to the lack of an appropriate disease model.

The discovery of induced pluripotent stem cells (iPSCs) in 2006 by Sinya Yamanaka started a revolution in biomedical research [6-7]. iPSCs can be generated reprogramming somatic cells only by the ectopic expression of four transcription factors (OCT3/4, SOX2, KLF4 and C-MYC). The resulting cells present similar molecular and functional characteristics as embryonic stem cells, enabling their directed differentiation into any cell type, such as RGCs.

In the last years, researchers have made great improvements in CRISPR/Cas9 editing technique. Using a specific RNA guide in combination with Cas9, the correction of any mutation turns out to be feasible, allowing us to generate isogenic iPSC controls [8]. Indeed, patient-derived iPSCs and its related isogenic controls would potentially be differentiated into the target cell type to search for the pathophysiological mechanisms of the disease.

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The main objective of this work has been the use of induced pluripotent stem cell (iPSC) technology as a tool for the generation of patient-specific iPSC-derived RGCs, the affected target cell type in patients with DOA. For that purpose, we have used an iPSC line previously created by reprogramming fibroblasts carrying the pathogenic variant c.1861C>T; p.Q621* in the *OPA1* gene. Moreover, our aim has been to correct the causative mutation in *OPA1* using CRISPR/Cas9 tool and differentiate both iPSC lines, the mutant and the corrected one into RGCs. These generated RGCs would allow to closely model DOA, opening up the possibility to identify an appropriate treatment.

2. Generation of the Isogenic Control Using CRISPR/Cas9 System

In this work, we have used an iPSC line, previously created in our laboratory, from fibroblasts obtained from a DOA 'plus' patient carrying the pathogenic variant c.1861C>T; p.Q621* in the *OPA1* gene [9]. Using the CRISPR/Cas9 genome editing tool this variant in the *OPA1* gene has been corrected in the patient-derived iPSCs.

For that purpose, ribonucleoprotein complexes including synthetic guide RNAs have been employed, in combination with a single-stranded DNA oligonucleotide template [10]. Following this protocol, 96 clones were manually isolated and, after *Restriction Fragment Length Polymorphism* (RFLP) analysis, 15,25% edition efficiency has been obtained. Then, the clones that showed to be positive in the analyses has been sequenced by Sanger to confirm it.

One of these clones has been selected basing on morphological and growth criteria. Subsequently, a complete battery of tests was performed in order to confirm the pluripotency and integrity of the edited line. The iPSC line showed expression of pluripotency markers, such as SOX2, OCT4, NANOG or C-MYC, which was verified by immunocitochemistry and real-time PCR. It was able to generate the three germ layers, endoderm, mesoderm and ectoderm (as shown by α-fetoprotein, β-III-tubulin and smooth muscle actin expression). Using DNA fingerprinting analysis, we demonstrated the line to have the same origin as the previously reported iPSCs [11]. The line was also mycoplasma negative and exhibited a normal karyotype.

3. iPSC Differentiation towards an RGC Lineage

We have differentiated both DOA iPSCs and the corrected iPSCs into RGCs using an stepwise protocol [12]. It consists on the addition of several small molecules to activate and repress different pathways in order to mimic the embryonic development. For this purpose, embryoid bodies are generated in suspension, being transferred to Matrigel for the formation of neural rosettes. Afterwards, retinal progenitor cells are isolated from these structures and further differentiated to RGCs in poly-D-lysine and laminin.

The generated RGCs showed expression of typical RGC markers, such as BRN3A, SNCG or THY1, both in immunocytochemistry and RT-PCR.

4. Conclusions and Future Perspectives

In this study, an isogenic RGC model of DOA 'plus' disease have been successfully generated. This model will be very useful to understand the pathophysiological mechanisms underlying DOA, as well as to be used as a platform to search for a potential treatment.

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Institutional Review Board Statement: The study was approved by the Ethics Committee of the 'Hospital Universitario 12 de Octubre' (Madrid, Spain) (protocol 21/482) and was performed in accordance with the Declaration of Helsinki for Human Research.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are available from the corresponding author upon reasonable request.

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