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Research Idea

Transmission Mechanism of Lewy Body-Like α-Synucleinopathies in Dopaminergic Neurons Derived from Human Induced Pluripotent Stem Cells

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Abstract

Grafting of cells in Parkinson's disease (PD) results in a prion-like infection, exhibiting a Lewy body-like pathology, caused by the recipient cells. The transmission mechanism of Lewy bodies is not completely understood. Therefore, a research idea with a novel experimental strategy is proposed to investigate the transmission mechanism of α-synuclein pathology using PD patient-derived human induced pluripotent stem cells (hiPSC) in an *in vitro* human cellular and molecular PD model and *in vivo* mouse PD model for dopaminergic neuron transplantation.

Keywords

Parkinson's disease, transplantation, cell therapy, alpha-synuclein, iPSC

Introduction

In recent years, cell therapy is highly anticipated as a valid Parkinson's disease (PD) treatment method in translational regenerative medicine; for example, induced pluripotent stem cell (iPSC)-derived dopaminergic (DA) neurons transplanted in the midbrain of a primate model of PD attenuated the parkinsonian symptoms Kikuchi et al. 2011. However, embryonic nigral transplantation in PD patients resulted in a prion-like infection, in that, the recipient cells promoted the donor cell-Lewy body-like pathology Kordower et al. 2008. To investigate the transmission mechanism, iPSCs derived from PD patients and mutation correction using CRISPR/Cas9 provide a fundamental disease model, with minimum genetic interference for immunohistochemical analysis and genome-wide epigenetic sequencing.

Method

Selection Criteria for PD patients

The parkinsonian symptoms are associated with various gene mutations such as *Parkin* and *SNCA Kalinderi et al. 2016*; in this case, missense mutated *SNCA*-encoded α-synuclein protein causes Lewy body-like pathology, which can also be identified in post-grafted nigral cells 14 years after transplantation Kordower et al. 2008. Therefore, PD patients with missense mutated *SNCA* i.e., Ala53Thr, Glu46Lys, His50Gln, Gly51Asp, and Ala30Pro are selected as donors of adult human dermal fibroblasts (HDF) for hiPSC generation Kim et al. 2014.

Induction of hiPSC

The acquired HDF are processed using the protocol of retrovirus-mediated transfection with Yamanaka 4 factors i.e., Oct3/4, Sox2, c-Myc, and Klf4, for reprogramming of the iPSCs Takahashi et al. 2007.

PD Mutation Correction via CRISPR/Cas9 and Single Cell Cloning

To circumvent the complications due to genetic background variation in PD patient-derived iPSCs, CRISPR/Cas9 genetic editing is performed to efficiently correct patient-specific disease mutations, i.e., in *SNCA*, involving Ala53Thr, Glu46Lys, His50Gln, Gly51Asp, and Ala30Pro. This strategy provides a single, different genetic background to the PD patient-derived iPSCs Kim et al. 2014. Once the genetic editing is complete, the cell density is diluted to achieve 1 cell per well (96 well plate) for identifying whether the colony from the single cell line is successfully corrected.

Induction of DA Neurons

To induce the differentiation of the iPSCs into DA neurons, the procedure used by Dr. J. Takahashi's team is followed Kikuchi et al. 2011.

Co-Culturing DA Neurons with Mis-Folded α-Synuclein

To understand the mechanism of endogenous α-synuclein transmission, both, the gene-corrected and PD patients' iPSC-derived DA neurons are co-cultured with the mis-folded (missense mutated) and wildtype α-synuclein that are labeled with GFP. The mis-folded α-synuclein may pass through the cell membranes and subsequently cause epigenetic changes.

Animal Model for DA Neuron Transplantation/Brain Slicing

Missense mutated *SNCA*, Ala53Thr, Glu46Lys, His50Gln, Gly51Asp, and Ala30Pro knock-in mice separately serve as in vivo PD animal models for transplantation of the GFP-labeled hiPSC-derived DA neurons into the substantia nigra. Mice are fed with anti-immune drugs after grafting. At the first week, second week, and first month post-transplantation, brain section of mid-brain substantia nigra is anatomically sliced.

Fluorescence/Immunocyto- or Histo-chemical Staining

1. Co-Culturing with mis-folded α-synuclein: After co-culturing and transplantation, the DA neurons are isolated and the protein location of the mis-folded α-synuclein is identified to whether it entered through the cell membrane or modified the epigenetics. With the mis-folded α-synuclein labeled with GFP, new mis-folded or wildtype α-synuclein translation is observed with immunocytochemical staining.
2. hiPSC-derived DA Neuron Transplantation: GFP-labeled post-grafted DA neurons can be located in the brain section of mid-brain substantia nigra. With immunohistochemical staining for α-synuclein, the phenomenon of transmission of endogenous Lewy body-like α-synucleinopathies is evaluated.

Epigenetic Genomic Sequencing

If mis-folded α-synuclein enters the cells and binds with the genome, it may alter the epigenetics and subsequently result in abnormal expression of the mRNAs or of other associated genes.

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