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Short communication

Cytoplasmic aggregates of dynactin in iPSC-derived tyrosine hydroxylase-positive neurons from a patient with Perry syndrome

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ABSTRACT

Background: Perry syndrome is a rare autosomal dominant disorder clinically characterized by parkinsonism with depression/apathy, weight loss, and central hypoventilation. Eight mutations in *DCTN1* gene have been reported. A novel disease model is required because the detailed pathogenesis remains unclear.
Methods: To develop a novel model, we generated induced pluripotent stem cells (iPSCs) from a Perry syndrome patient with F52L mutation in *DCTN1*, and describe clinical and neuroimaging investigations. We differentiated iPSCs into tyrosine hydroxylase (TH)-positive neurons. Immunocytochemistry analyses of control and mutant were performed.
Results: The patient displayed levodopa responsive parkinsonism. Dopamine transporter single photon emission tomography showed markedly decreased uptake in the striatum, and meta-iodobenzylguanidine cardiac scintigraphy also showed decreased uptake. Perry syndrome TH-positive neurons showed dynactin aggregates in cytoplasm.
Conclusions: TH-positive neurons from Perry syndrome iPSCs recapitulated an aspect of the disease phenotype of Perry syndrome.

1. Introduction

Perry syndrome is a rare genetic disorder associated with parkinsonism, depression/apathy, weight loss, and central hypoventilation [1]. Histology showed neuronal loss and gliosis in the substantia nigra, and transactive response DNA-binding protein 43 (TDP-43) and dynactin-positive inclusions in the neurons of the basal ganglia and brainstem including substantia nigra [1]. Eight point mutations have been identified: F52L, G71A, G67D, G71R, G71E, T72P, Q74P, and Y78C located in exon 2 of *DCTN1*. The clinical presentations caused by each mutation are almost the same, but F52L mutation occurs later in the disease course [2]. In this study, we successfully obtained induced pluripotent stem cells (iPSCs) from a patient with F52L and differentiated them into tyrosine hydroxylase (TH)-positive neurons.

2. Methods

2.1. Participant

The subject of this study was a Japanese man whose genetic presentation has already been briefly described. He was a IV-2 patient in a previous report [2]. He provided written informed consent. We report the clinical course and imaging study of this

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patient.

2.2. Standard protocol approval

This study was approved by the Institutional Review Boards of Kyoto University and Fukuoka University.

2.3. Generation of iPSCs and TH-positive neuron differentiation

Peripheral blood mononuclear cells were reprogrammed by inducing the episomal vectors carrying OCT3/4, SOX2, KLF4, L-MYC, LIN28, EBNA1, and p53 carboxy-terminal dominant-negative fragment [3]. We used a feeder-free culture system to establish iPSCs [4]. We produced control iPSCs from an unrelated healthy individual and Perry syndrome patient iPSCs. We characterized TH-positive neurons by quick embryoid body–like aggregate method [5] and dual SMAD inhibition by modifying previous procedures [6]. Briefly, a floating culture of cell aggregates was introduced, and LDN193189, A83-01, purmorphamine, CHIR99021, fibroblast growth factor 8, brain-derived neurotrophic factor, glial cell-derived neurotrophic factor, dibutyryl cyclic AMP, and ascorbic acid were added. TH-positive neurons were evaluated 6–9 weeks later *in vitro*.

2.4. HEK293T cells with of $p150^{glued}$ -GFP overexpression

Dynactin consists of many subunits, with $p150^{glued}$ protein, encoded by *DCTN1* gene, being one of them. HEK293T cells were transiently transfected with GFP-tagged wild-type (WT) and mutant F52L $p150^{glued}$ (c.156T > A) using Lipofectamine LTX (Thermo Fisher Scientific) according to the manufacturer's protocol. Analysis was performed 24 h after transfection.

2.5. Immunocytochemistry

HEK293T cells transfected with GFP-tagged WT and mutant F52L $p150^{glued}$, and TH-positive neurons derived from the control and patient iPSCs were assessed by immunocytochemistry.

Cells were fixed with 4% paraformaldehyde and blocked with PBS containing 5% fetal bovine serum or Blocking One Histo (Nacalai Tesque). DAPI (4′, 6-diamidino-2-phenylindole) (Life Technologies) was used to label nuclei. Fluorescence imaging was performed using BIOREVO (Keyence), IN CELL Analyzer 6000 (GE Healthcare), and Delta Vision (Applied Precision). The following primary antibodies were used: NANOG (REPROCELL, 1:500), SSEA-4 (Millipore, 1:1,000), SOX-17 (R&D Systems, 1: 300), αSMA (DAKO, 1:3,000), Tuj1 (Covance, 1:2,000), TH (Millipore, 1: 600), $p150^{glued}$ (abcam, 1:200), TDP-43 (Proteintech, 1:250), ubiquitin (DAKO, 1:1,000), α-tubulin (SIGMA-ALDRICH, 1:1,000), p50 (Santa Cruz Biotechnology, 1:50), p62 (Santa Cruz Biotechnology, 1:50), TOM20 (Santa Cruz Biotechnology, 1:50), LAMP2 (abcam, 1:100), Calnexin (Enzo Life Sciences, 1:200). We quantified the number of $p150^{glued}$ aggregate-positive cells in HEK293T or iPSC-derived TH-positive neurons. We defined the $p150^{glued}$ aggregates as fluorescent accumulations, observed in the cytosol of iPSC-derived TH-positive neurons by using Z-stacked slices. Detection of aggregates was performed blindly. Results represent quantitation from randomly selected fields for each mutation or clone in a blinded manner. Results are representative of three experiments for HEK293T cells or four experiments for iPSC-derived TH-positive neurons.

2.6. Statistics

T-test was performed. Significance was set at $P < 0.05$.

3. Results

3.1. Case report

The patient (Fig. 1A) was a 61-year-old man. He had a family history of parkinsonism in his mother and aunt. He presented with depression at the age of 53. At age 55, he developed progressive bradykinesia, walking difficulty and mild tremor in his hands. At that time, neurological examination revealed bradykinesia, rigidity, postural instability, and postural tremor in his hands. He was treated with levodopa/carbidopa, which provided moderate benefit. Subsequently, he experienced unexpected weight loss and constipation. The patient's cognition was normal during the follow-up of two years, with a Mini-Mental State Examination score of 29/30 at age 57. On admission, the patient's postural instability had profoundly deteriorated. Brain MRI showed mild frontotemporal atrophy (Fig. 1C). ^{123}I-metaiodobenzylguanidine cardiac scintigraphy showed decreased uptake (heart/mediastinum [H/M] ratio: 1.82 early phase and 1.65 late phase) (Fig. 1B). ^{123}I-FP-CIT–single photon emission computed tomography revealed markedly low uptake (Fig. 1D) in the striatum (specific binding ratio: Rt = 0.27 and Lt = 0.11).

3.2. Generation of iPSC and TH-positive neuron differentiation

One clone from control iPSCs and one clone from the patient iPSCs were analyzed. These iPSCs exhibited morphology similar to human embryonic stem cells and were positive for NANOG and SSEA4 staining (Fig. 1E). These iPSCs were able to differentiate into cells of all three germ layers *in vitro* (Fig. 1F) and had normal karyotypes (Fig. 1G). Genomic analysis showed the presence of a point mutation in *DCTN1* only in Perry syndrome iPSCs (Fig. 1H).

To confirm that cells derived from patient iPSCs and control were TH-positive neurons, we performed immunofluorescence staining with both TH and Tuj1. The Tuj1-positive cells coexpressed TH (Fig. 1I).

3.3. Cellular distribution of $p150^{glued}$ in control and F52L

Histological studies of postmortem brain tissue have shown dynactin and TDP-43-positive inclusions in Perry syndrome. To recapitulate Perry syndrome pathology [1], we first used HEK293T cells transiently transfected with GFP-tagged WT and mutant F52L $p150^{glued}$. HEK293T overexpressing GFP-tagged WT $p150^{glued}$ showed thread-like cytoplasmic distribution. By contrast, those with GFP-tagged mutant F52L $p150^{glued}$ showed diffuse cytoplasmic distribution with various sizes of aggregates (Fig. 2A, B, E, G, Supplementary Fig. 1A, D, Supplementary Fig. 2A, C). We next analyzed control and Perry syndrome iPSCs and TH-positive neurons derived from each of the iPSCs. We provided positive control of $p150^{glued}$ antibody using HEK293T cells (Supplementary Fig. 1A). We detected various sizes of $p150^{glued}$ aggregates in cytoplasm and neurites only in Perry syndrome patient iPSC-derived TH-positive neurons (Fig. 2C, D, F, H, Supplementary Fig. 1B, E, Supplementary Fig. 2B, D). Control and Perry syndrome patient iPSCs did not present aggregates (Supplementary Fig. 1C). In both HEK293T cells and TH-positive neurons, $p150^{glued}$ aggregates were not positive for TDP-43 (Fig. 2E, F). We examined the characteristics of the aggregates by immunocytochemistry. A small proportion of aggregates of the Perry syndrome patient iPSC-derived TH-positive neurons was partially positive for ubiquitin but not for those of HEK293T overexpressing GFP-tagged F52L $p150^{glued}$ (Fig. 2G, H). p50 and p62, other subunits of dynactin, partially co-localized with $p150^{glued}$ aggregates in HEK293T cells and TH-positive neurons (Supplementary Fig. 1D, E). We conducted additional staining