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Lab resource: Stem Cell Line

Generation of an induced pluripotent stem cell line (GIBHi004-A) from a Parkinson's disease patient with mutant DJ-1/PARK7 (p.L10P)

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A B S T R A C T

Mutations occurring in the gene body of *PARK7* (encoding DJ-1/PARK7) cause autosomal recessive early-onset parkinsonism (AREP). These mutations produce a loss of function and have been reported to lead to dopaminergic neuron degeneration in the substantia nigra. However, the underlying mechanisms are largely unknown. Here, we report the generation of a patient-derived induced pluripotent stem cell (iPSC) line carrying mutant DJ-1 (p.L10P). This cell line is a valuable tool for *in vitro* Parkinson's disease (PD) modeling and mechanistic studies.

1. Resource table

Unique stem cell lines identifier	GIBHi004-A
Alternative names of stem cell lines	PARK7-L10P-C2
Institution	Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China
Contact information of distributor	Wenjuan Li, e-mail: li_wenjuan@gibh.ac.cn
Type of cell lines	iPSCs
Origin	Human
Additional origin information	Age: 23 Gender: Male Ethnicity: Chinese
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	Retroviral *OCT4, SOX2, KLF4, MYC*
Gene modification	Yes
Type of modification	Hereditary
Associated disease	Parkinson's disease
Gene/locus	*PARK7* gene/Chromosome 1p36.23, c.29 T > C (p.L10P)
Method of modification	*N/A*
Name of transgene or resistance	*N/A*
Inducible/constitutive system	*N/A*
Date archived/stock date	May 2018
Cell line repository/bank	https://hpscreg.eu/cell-line/GIBHi004-A
Ethical approval	The Ethics Committee of the Guangzhou Institutes of Biomedicine and Health (Guangzhou, China) approved this procedure (approval no. 2014003)

2. Resource utility

To date, the mechanisms by which *PARK7* mutations cause dopaminergic neuron degeneration linked to AREP are still unclear. Here, we have generated an iPSC clone with mutant DJ-1 (p.L10P) from patient-derived fibroblasts. This cell line will facilitate disease modeling and mechanistic studies of PD *in vitro*.

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3. Resource details

Human *PARK7* is one of the genes mutated in AREP. DJ-1 has a role in protecting cells from oxidative stress (Meiser et al., 2016). Several missense mutations in DJ-1, including the L166P, M26I, L10P, and P158D, disrupt the formation of DJ-1 homodimers, causing poor folding and proteolytic degradation of the protein (Zeng et al., 2018). Among these mutations, it has been reported the case of a patient carrying familial DJ-1 L10P mutation that led to the development of typical PD symptoms at the age of 18 (Guo et al., 2008).

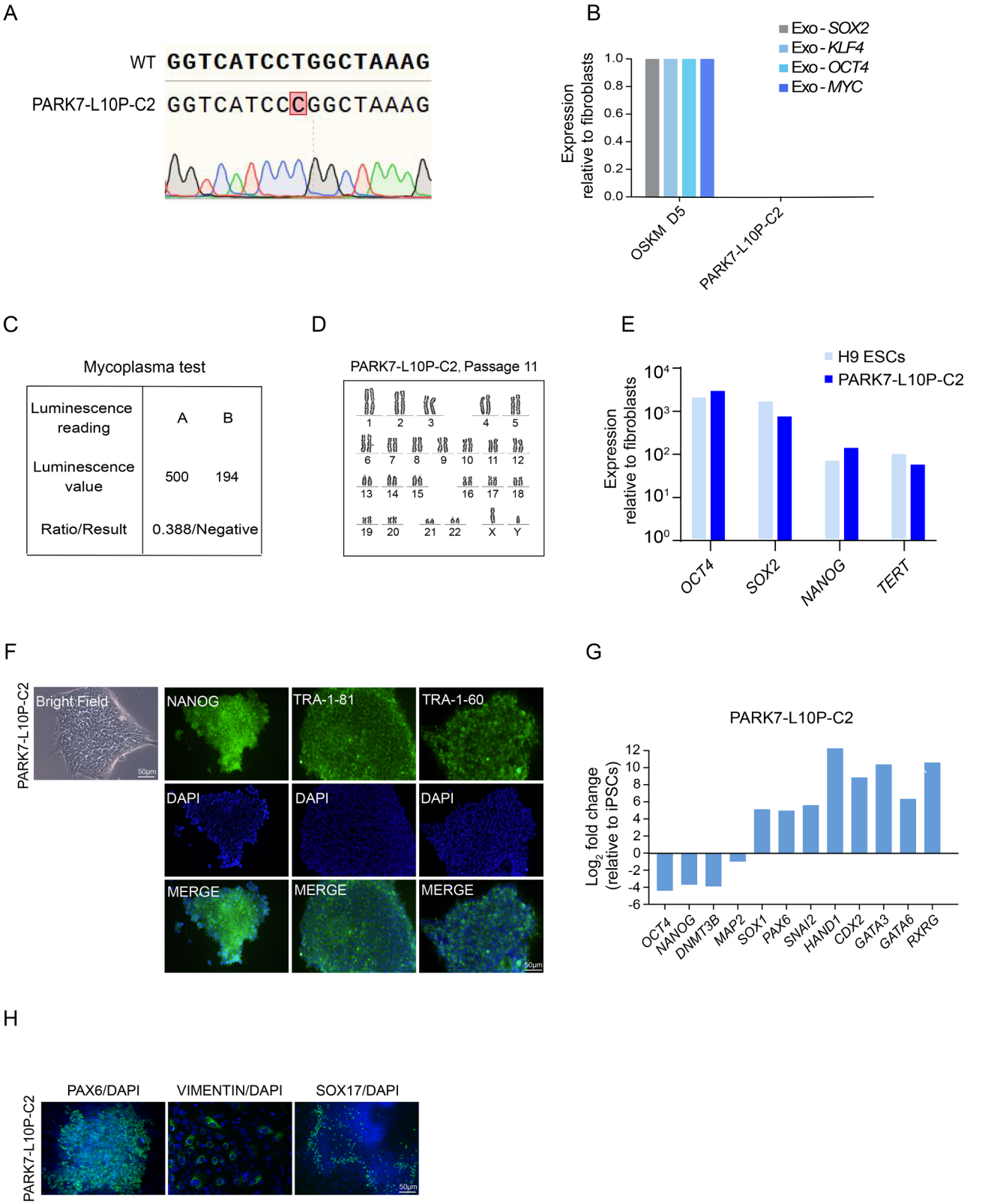

Fig. 1. Characterization of DJ-1/PARK7-L10P (GIBHi004-A) iPSC clone.

Patient-derived iPSCs can be readily differentiated into disease-related cell types such as dopaminergic neurons that can be subsequently used for disease modeling *in vitro* (Zhao et al., 2014). Using skin fibroblasts of PD patient with homozygous missense mutation in *PARK7* (c.29 T > C), we generated an iPSC clone (PARK7-L10P-C2) by retroviral overexpression of *OCT4*, *SOX2*, *KLF4*, and *MYC*. The *PARK7* mutation in this iPSC clone was verified by PCR and Sanger sequencing (Fig. 1A). Following this, we confirmed that exogenous *OCT4*, *SOX2*, *KLF4*, and *MYC* retroviruses were silenced by reverse transcription quantitative PCR (RT-qPCR) (Fig. 1B). Additionally, this clone was free from mycoplasma contamination and had normal karyotype (Fig. 1C and D). Short tandem repeat (STR) assay results also confirmed that it was indeed derived from the same donor fibroblasts. Next, the expression of endogenous pluripotency genes *OCT4*, *SOX2*, *NANOG* and *TERT* were validated at the mRNA level by RT-qPCR (Fig. 1E). As expected, we observed typical human embryonic stem cell-like morphology and immunostaining for pluripotency markers NANOG, TRA1-60, and TRA1-81 was positive (Fig. 1F). Finally, embryoid body (EB) differentiation followed by RT-qPCR detected robust induction of ectoderm (*MAP2*, *SOX1*, and *PAX6)*, mesoderm (*SNAI2*, *HAND1*, and *CDX2)*, and endoderm (*GATA3*, *GATA6*, and *RXRG)* (Fig. 1G), whereas immunostaining of attached EBs showed the presence of cells positive for PAX6 (ectoderm), VIMENTIN (mesoderm), and SOX17 (endoderm) (Fig. 1H).

In conclusion, this well-characterized iPSC clone will be useful for the *in vitro* disease modeling of PARK7-related AREP and accelerate mechanistic studies (See Table 1).

4. Materials and methods

4.1. Cell culture

Fibroblasts from an early-onset PD patient with mutant DJ-1 were cultured in DMEM (CORNING, 07719005) supplemented with 10% FBS (NATOCOR, NTC-HK023), 1% non-essential amino acids (Gibco, 11140050), 1% Glutamax (Gibco, 35050079), 1% sodium pyruvate (CORNING, 24118006), and 1% penicillin–streptomycin (Hyclone, J190014). Reprogramming was performed using retroviral plasmids (Addgene) producing human OCT4, SOX2, KLF4, and MYC transcription factors. HEK293T cells were transfected with individual retroviral plasmid together with the packaging plasmids. 48 h after transfection, viral supernatants were harvested on two consecutive days. Fibroblasts were trypsinized and seeded as 40,000 cells/well on 6-well plates. They were infected with viral supernatants supplemented with 8 μg/ml polybrene (Sigma). Then, transduced cells were fed daily with human ESC medium: DMEM/F12, 20% KSR (Thermo Fisher Scientific, A3181502), 10 ng/ml basic fibroblast growth factor (NOVUS, NBP2-34921), 1% non-essential amino acids (Gibco, 11140050), 1% Glutamax (Gibco, 35050079), and 0.1 mM β-mercaptoethanol. Four days later, 1 mM valproic acid (Sigma, P4543) and 50 μg/ml ascorbic acid (Sigma, A5960) were added into the medium for another 8–15 days to boost reprogramming efficiency. Then, valproic acid and ascorbic acid were withdrawn, and colonies with typical human ESC-like morphology were picked and adapted to a feeder free culture condition (mTeSR1/Matrigel). Picked colonies were cultured on Matrigel (Corning, 354277)-coated plates in mTeSR1 medium (Stemcell, 85850) and routinely passaged every 3–4 days with 0.5 mM EDTA. Incubation conditions were 37 °C and normoxia with 5% CO_2. CryoStor® cell cryopreservation media CS10 (Sigma, C2874) and 10 μM ROCK inhibitor Y27632 (Selleck, S1049) were used for cryopreservation of iPSCs.

4.2. RT-qPCR

Total RNA was prepared with RNAzol® RT (MRC, RN 190) and converted to cDNA using oligo$(dT)_{20}$-primers from ReverTra Ace-α-® kit (TOYOBO). RT-qPCR was performed using SYBR Green (Takara, RR420A) and an ABI 7500 real-time PCR machine. Data were analyzed based on 2^ΔΔCt method. Primer sequences are listed in Table 2.

4.3. Immunofluorescence

Cells were fixed with 4% paraformaldehyde at room temperature for 10 min and permeabilized and blocked with 0.2% Triton X-100 containing 3% BSA at room temperature for 1 h. Then, they were incubated with primary antibodies diluted in 3% BSA at 4 °C overnight. After washing three times with PBS, they were incubated with secondary antibodies diluted in 3% BSA at room temperature for 1 h. Finally,

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel F
Phenotype	Qualitative analysis (Immunocytochemistry)	Immunostaining of pluripotency markers: NANOG, TRA-1-60, and TRA-1-81	Fig. 1 panel F
	Quantitative analysis (RT-qPCR)	RT-qPCR for *OCT4, SOX2*, *NANOG*, and *TERT*	Fig. 1 panel E
Genotype	Karyotype (G-banding) and resolution	PARK7-L10P-C2: 46, XY Resolution: 400	Fig. 1 panel D
Identity	Microsatellite PCR (mPCR)	N/A	N/A
	STR analysis	STR: 20 sites tested and all matched	Available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	Confirmed genotypes: Homozygous mutation PARK7-L10P-C2: c.29 T > C	Fig. 1 panel A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. Negative	Fig. 1 panel C
Differentiation potential	Embryoid body formation OR Teratoma formation OR Scorecard OR Directed differentiation	Expression of three germ layers marker genes in EB formation experiments: Ectoderm: *MAP2*, *SOX1*, and *PAX6* Mesoderm: *SNAI2*, *HAND1*, and *CDX2* Endoderm: *GATA3*, *GATA6*, and *RXRG*Immunofluorescence of three germ layers markers in EB formation: Ectoderm: PAX6 Mesoderm: VIMENTIN Endoderm: SOX17	Fig. 1 panel G Fig. 1 panel H
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

Table 2
Reagents details.

Antibodies used for Western blotting/immunocytochemistry			
	Antibody	Dilution	Company Cat# and RRID
Pluripotency markers (immunocytochemistry)	Rabbit anti-NANOG	1:200	Abcam Cat# ab21624, RRID: AB_446437
	Mouse anti-TRA-1–60	1:100	Merck Millipore Cat# MAB4360, RRID: AB_2119183
	Mouse anti-TRA-1–81	1:50	Merck Millipore Cat# MAB4381, RRID: AB_177638
Differentiation markers (immunocytochemistry)	Mouse anti-VIMENTIN	1:200	Sigma Cat# v5255, RRID: AB_477625
	Goat anti-SOX17	1:200	R&D Systems Cat# AF1924, RRID: AB_355060
	Mouse anti-PAX6	1:250	DSHB Cat# PAX6, RRID: AB_528427
Secondary antibodies	Alexa Fluor 488 Goat anti-Mouse IgG	1:500	Invitrogen Cat# A21141, RRID: AB_141626
	Alexa Fluor 488 Goat anti-Mouse IgM	1:400	Invitrogen Cat# A21042, RRID: AB_2535711
	Alexa Fluor 488 Rabbit anti- Goat IgG	1:400	Invitrogen Cat# A11078, RRID: AB_2534122
Primers			
	Target		Forward/Reverse primer (5′-3′)
Pluripotency Markers (qPCR)	*OCT4*		CCTCACTTCACTGCACTGTA/CAGGTTTTCTTTCCCTAGCT
	SOX2		CCCAGCAGACTTCACATGT/CCTCCCATTTCCCTCGTTTT
	NANOG		TGAACCTCAGCTACAAACAG/TGGTGGTAGGAAGAGTAAAG
	TERT		ACGCTTCATGTGCCACCA/TTCTCCATGTCGCCGTAGC
	DNMT3B		AGGGAAGACTCGATCCTCGTC/GTGTGTAGCTTAGCAGACTGG
Differentiation Markers (qPCR)	*MAP2*		TGGTGCCGAGTGAGAAGAAG/AGTGGTTGGTTAATAAGCCGAAG
	SOX1		CCTCCGTCCATCCTCTG/AAAGCATCAAACAACCTCAAG
	PAX6		TCTTTGCTTGGGAAATCCG/CTGCCCGTTCAACATCCTTAG
	SNAI2		CAGACCCTGGTTGCTTCAA/TGACCTGTCTGCAAATGCTC
	HAND1		CCAAGGATGCACAGTCTGG/CGGTGCGTCCTTTAATCCT
	CDX2		CTGGAGCTGGAGAAGGAGTTTC/ATTTTAACCTGCCTCTCAGAGAGC
	GATA3		TGTGTGAACTGTGGGGCAA/TCTGACAGTTCGCACAGGAC
	GATA6		ACTTGAGCTCGCTGTTCTCG/CAGCAAAAATACTTCCCCCA
	RXRG		AGCAGCTTTTCACCCTCGT/ACATGTAAACCCGTGGCCA
House-Keeping Genes (qPCR)	*ACTB*		CCCAGAGCAAGAGAGG/GTCCAGACGCAGGATG

nuclei were stained with 5 ng/ml DAPI as counterstaining, followed by imaging using a ZEISS Axio Vert.A1 microscope. Primary antibodies used for immunofluorescence are listed in Table 2.

4.4. STR analysis

DNA were extracted using Wizard® Genomic DNA Purification Kit (Promega, A1120). STR analysis was performed using AmpFLSTR Identifiler PCR Amplification Kit (ThermoFisher Scientific, 4322288) following manufacturer's instructions.

4.5. Karyotyping

Karyotyping was performed in cells at passage 11 by G-banding. Briefly, cells were treated with 100 ng/ml colcemid for 1 h for metaphase arrest. Arrested cells were trypsinized, pelleted, and resuspended with 100 μl PBS followed by addition of 10 ml hypotonic solution (0.56 g KCl and 0.5 g sodium citrate in 200 ml H_2O) dropwise. Finally, cells were fixed with fixation buffer (3 parts methanol to 1 part acetic acid). A total of 20 randomly selected metaphase spreads were counted and stained using standard G-banding analysis at 400-band resolution.

4.6. Mycoplasma detection

Mycoplasma detection was performed using MycoAlert™ Mycoplasma Detection Kit (LONZA, LT07-318) following manufacturer's instructions. Stability of the assay was verified by positive and negative controls provided in the kit.

4.7. EB differentiation

iPSCs were first dissociated with Accutase (Sigma, A6964) to single cells, then 1.5 million cells were resuspended in EB differentiation medium: KnockOut™ DMEM (Thermo Fisher Scientific, 10829018), 1% Glutamax, 1% non-essential amino acids, and 20% KSR with 10 μM Y27632, and transferred to a well of an AggreWell™ 800 plate (Stemcell, 34811) and cultured for 24 h to form EBs. These EBs were transferred to a low adherent dish and continuously cultured in EB differentiation medium for 15 days, with the medium changed every 3 days. Then, RNA was extracted to check expression of different differentiation markers. EBs for immunostaining were attached to Matrigel-coated plates at day 10 and were fixed at day 15.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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