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Lab resource: Stem Cell Line

Generation of an induced pluripotent stem cell line from a patient with retinitis pigmentosa caused by *RP1* mutation

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A B S T R A C T

We report the generation of the iPSC line LEIi005-B from a patient with retinitis pigmentosa caused by a dominant nonsense mutation in the *RP1* gene (c.2098G > T p.E700X). Reprogramming of dermal fibroblasts was performed using episomal plasmids containing *OCT4*, *SOX2*, *KLF4*, *L-MYC*, *LIN28*, mir302/367 microRNA and shRNA for *p53* to establish the clonal iPSC line LEIi005-B. LEIi005-B expressed pluripotent stem cell markers, had a normal karyotype and differentiated into endoderm, mesoderm and ectoderm.

Resource table.

Unique stem cell line identifier	LEIi005-B
Alternative name of stem cell line	RP1ips6
Institution	The University of Western Australia
Contact information of distributor	Dr Samuel McLenachan smclenachan@lei.org.au Dr. Fred K Chen fredchen@lei.org.au
Type of cell line	iPSC
Origin	Human
Additional origin info	76 year old Caucasian female
Cell Source	Dermal fibroblasts
Clonality	Clonal
Method of reprogramming	Episomal Vectors
Genetic Modification	No
Type of Modification	N/A
Associated disease	Retinitis pigmentosa
Gene/locus	*RP1*/8q11.23-q12.1
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	01/08/2017
Cell line repository/bank	N/A
Ethical approval	Human Research Ethics Office, University of Western Australia (RA/4/1/7916)

Resource utility

Mutations in the *RP1* gene are a common cause of autosomal dominant retinitis pigmentosa. The LEIi005-B iPSC line was generated from a patient with retinitis pigmentosa caused by a nonsense mutation in the *RP1* gene (c.2098G > T p.E700X). This line will provide valuable insights into the pathophysiological mechanisms of retinitis pigmentosa.

Resource details

Retinitis pigmentosa (RP) is a heterogeneous group of inherited retinal diseases, affecting 1 child in every 3000–7000 births (Audo et al., 2012). The most common mode of inheritance in RP is autosomal dominant (ad, up to 25% of RP) (Ferrari et al., 2011). One of the three most common genes causing adRP is *RP1* (up to 10% of adRP) (Sullivan et al., 1999). Here, we present an iPSC line generated from fibroblasts from a patient with adRP. The 76 year-old caucasian female patient developed nyctalopia from the age of 28. She had grossly delayed and reduced dark- and light-adapted full-field electroretinography (ERG) despite visual acuity of 6/9 and 6/12 in her right and left eyes, respectively, at the age of 38. Full-field ERG was essentially flat by the age of 44. The patient has a son with similar clinical features of extensive

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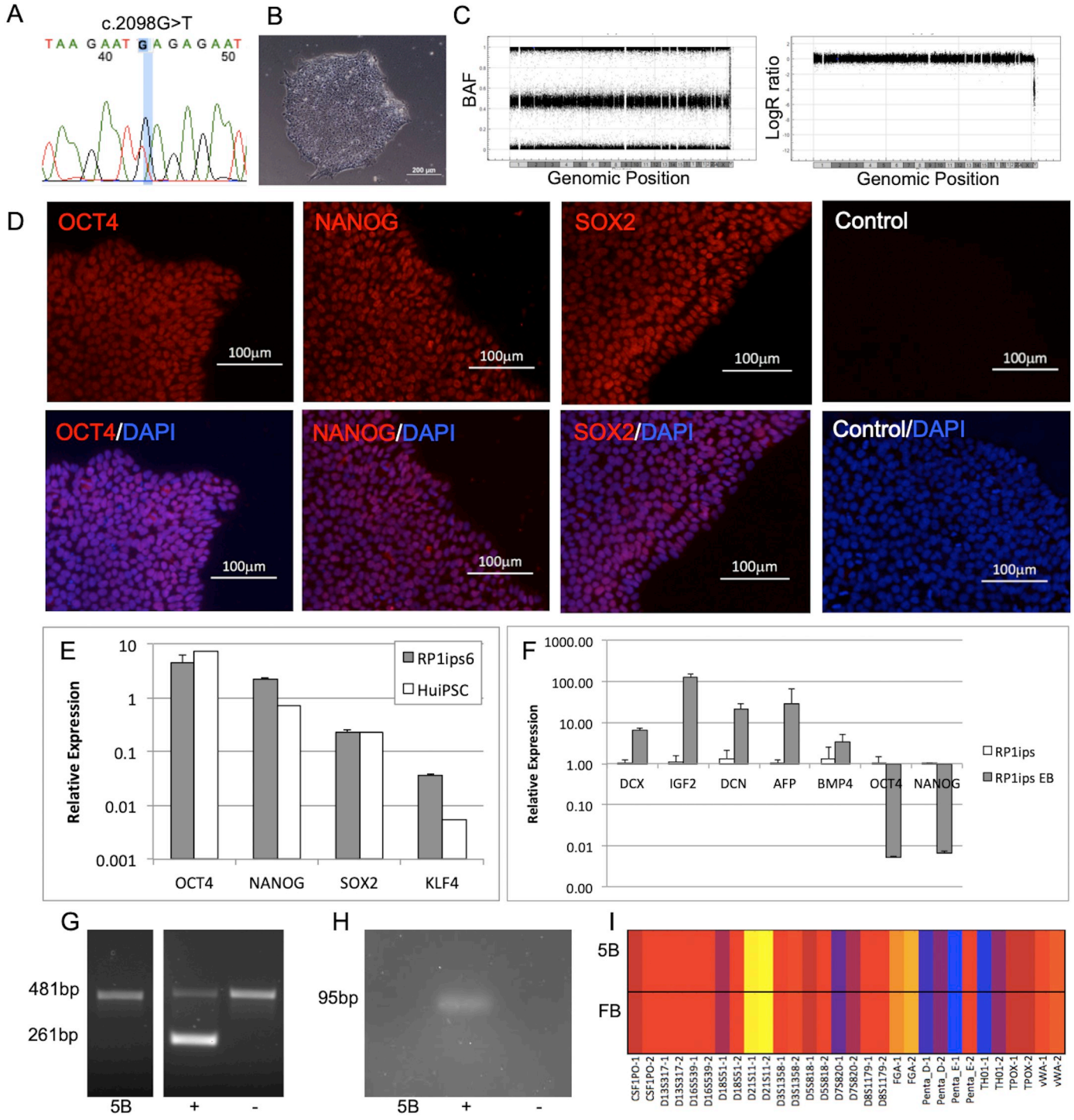

Fig. 1. A: Sanger sequencing demonstrated LEIi005-B iPSC are heterozygous for the c.2098G > T mutation. **B:** LEIi005-B colonies displayed typical pluripotent stem cell morphology. **C:** B-allele frequencies (BAF) and LogR ratios were plotted against genomic location for 500,000 human SNPs, demonstrating a normal, 46, XX karyotype in LEIi005-B. **D:** Immunocytochemistry analysis demonstrated the expression of pluripotency markers (OCT4, NANOG and SOX2) in LEIi005-B. Nuclei were counterstained with DAPI (merged images). Controls in which the primary antibody was omitted displayed no background signal. Scale bars indicate 100μm. **E:** Quantitative RT-PCR analysis demonstrated LEIi005-B (grey bars) and a commercial iPSC line (HuiPSC, white bars) expressed similar levels of the pluripotency markers *OCT4, NANOG, SOX2* and *KLF4*. **F:** Quantitative RT-PCR analysis demonstrated upregulation of ectodermal (DCX), mesodermal (IGF2, DCN, BMP4) and endodermal (AFP) markers in embryoid bodies generated from LEIi005-B (RP1ips EB), compared with undifferentiated LEIi005-B-iPSC (RP1ips). **G:** PCR screening demonstrated LEIi005-B (5B) was negative for mycoplasma. Mycoplasma positive (+) and negative (-) controls yielded bands of the expected sizes. **H:** Reprogramming episomes were detected by PCR (95bp band) in passage 1 iPSCs (+). No PCR products were amplified from passage 15 LEIi005-B iPSC genomic DNA (5B) or a negative control reaction without template DNA (-). **I:** Microsatellite analysis of LEIi005-B and the parental fibroblast line demonstrated identity at 15 loci, indicating both lines are derived from the same individual.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1B
Phenotype	Qualitative analysis: Immunocytochemistry	Positive for the pluripotency markers: OCT4, NANOG, SOX2	Fig. 1D
	Quantitative analysis: RT-qPCR)	Positive for the pluripotency markers *OCT4*, *NANOG*, *SOX2*, *KLF4*	Fig. 1E
Genotype	Illumina Beadchip SNP Array with CNV Analysis	46,XX 0.5MB resolution	Fig. 1C
Identity	STR analysis: PowerPlex 16HS System	Matched at 16 loci	Fig. 1I, Table submitted in archive with journal
Mutation analysis	Sequencing	*RP1* c.2098 G > T heterozygous nonsense mutation	Fig. 1A
Microbiology and virology	Mycoplasma - Lookout Mycoplasma PCR Detection Kit	Mycoplasma testing by PCR: Negative	Fig. 1G
Differentiation potential	Embryoid body formation: qPCR	Positive for trilineage markers *DCX*, *IGF2*, *DCN*, *AFP*, *BMP4*	Fig. 1F
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

peripheral retinal atrophy, bony spicules and arteriolar attenuation, consistent with RP. Both were subsequently found to be heterozygous for a nonsense mutation located in exon 4 (NM_006269.1: c.2098G > T p.(E700X)) of the *RP1* gene.

Dermal fibroblasts were cultured from the patient's skin biopsy sample. Reprogramming was performed on patient fibroblasts using episomal plasmids expressing *OCT4*, *SOX2*, *KLF4*, *L-MYC*, *LIN28*, *p53* shRNA, and miR-302/367. A clonal iPSC cell line (LEIi005-B) was established and characterized (Fig. 1, Table 1). The *RP1* c.2098G > T mutation was confirmed in the LEIi005-B iPSC line by Sanger sequencing (Fig. 1A). LEIi005-B iPSC displayed morphology typical of pluripotent stem cell colonies (Fig. 1B). Genome-wide copy number variation profiling demonstrated that the iPSC were female and had normal diploid karyotypes (46, XX). Analysis of the B allele frequencies (left panel) and LogR ratios (right panel) of 500,000 single nucleotide polymorphisms located across the genome demonstrated an absence of chromosomal rearrangements in LEIi005-B (Fig. 1C). The expression of the pluripotency markers OCT4, NANOG, and SOX2 was demonstrated by immunostaining analyses (Fig. 1D). Quantitative real time polymerase chain reaction (qPCR) analysis demonstrated similar levels of *OCT4*, *NANOG*, *SOX2* and *KLF4* expression in LEIi005-B and a control human iPSC line (HuiPSC, Fig. 1E). The potential of the LEIi005-B iPSC line to differentiate into three germ layers was demonstrated by the upregulation of markers of mesoderm (*IGF2*, *DCN*, *BMP4*), endoderm (*AFP*) and ectoderm (*DCX*) lineages in differentiating embryoid body (EB) cultures (Fig. 1F). The pluripotency genes *OCT4* and *NANOG* were downregulated in differentiating EBs (Fig. 1F). LEIi005-B was screened for mycoplasma using the Lookout Mycoplasma PCR Detection Kit. A 261 bp band was amplified from the positive control (+) but not the negative control (−) or LEIi005-B (5B). The 481 bp internal PCR control band was amplified from all samples (Fig. 1G). Reprogramming episomes could be detected as a 95 bp PCR product in passage 1 iPSCs (+) but were not detected in LEIi005-B after passage 15 (5B) or in negative control (−) containing no template DNA (Fig. 1H). Analysis of 15 microsatellite markers confirmed LEIi005-B (5B) was derived from the patient's fibroblasts (FB) (Fig. 1I).

Materials and methods

Ethics

Patient DNA was sourced and genetically analyzed by the Australian Inherited Retinal Disease Registry and DNA Bank (AIRDR), as approved by the Sir Charles Gairdner Hospital Human Research Ethics Committee (2001–053). Collection of patient samples and generation of iPSCs was approved by the University of Western Australia Human Research Ethics Committee (RA/4/1/7916). Written consent was obtained from the patient and all procedures were carried out in accordance with the requirements of the National Health & Medical Research Council of Australia and the Declaration of Helsinki.

Cell culture and differentiation

Patient fibroblasts were cultured in DMEM medium supplemented with 10% fetal calf serum (ThermoFisher). Patient iPSCs were cultured on Geltrex (ThermoFisher) coated culture plates in TeSR-E8 medium (Stem Cell Technologies). For EB differentiation, iPSC were seeded as cell clusters into uncoated culture plates in mTesR1 media (Stem Cell Technologies) supplemented with 10 μM Y27632 (Sigma-Aldrich). After 48 h, the media was changed to DMEM supplemented with 20% knockout serum replacement (KSR), non-essential amino acids and antibiotic-antimycotics (ThermoFisher). On day 14 of culture, RNA was harvested for qPCR analysis.

Reprogramming method

Patient's fibroblasts were reprogrammed using the Episomal iPSC Reprogramming Plasmid kit (SC900A-1, System Biosciences), according to the manufacturer's instructions. Colonies were picked for clonal expansion on day 25. The *RP1* c.2098G > T mutation was confirmed in both fibroblasts and iPSCs by PCR and Sanger sequencing. Primer sequences are listed in Table 2.

Quantitative Real-Time PCR analysis

Total mRNA was isolated using TRIZOL and cDNA was synthesized using the RT2 First Strand Kit (Qiagen). qPCR was performed using the CFX Connect Real-Time System (BioRad) and data analyzed using the ΔΔCT method. Gene expression values were normalized to *GAPDH* expression and expressed as mean expression relative to GAPDH (Fig. 1E) or as fold changes relative to undifferentiated iPSC (Fig. 1F). Primers used in qPCR assays are listed in Table 2.

Immunostaining analysis

Cells were cultured on Geltrex-coated coverslips, fixed with 4% paraformaldehyde, washed, then permeabilized using phosphate buffered saline (PBS) with 0.1% Triton X-100 for 15 min. The cells were then incubated in blocking buffer (5% BSA in PBS) for 1 h at room temperature. Primary antibodies were applied at 4 °C overnight.

Table 2
Reagents details.

Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Mouse anti-OCT4	1:200	Santa Cruz Biotechnology: sc-5279, RRID: AB_628051
	Rabbit anti-SOX2	1:200	Thermo Fisher Scientific: 48–1400, RRID: AB_2533841
	Rabbit anti-NANOG	1:100	Abcam: ab21624, RRID: AB_446437
Secondary antibodies	Alexa Fluor 546 Goat anti-mouse	1:500	Molecular Probes: A-11003, RRID:AB_141370
	Alexa Fluor 488 Goat anti-rabbit	1:500	Molecular Probes: A-11008, RRID:AB_143165

Primers		
	Target	Forward/Reverse primer (5′-3′)
Pluripotency Markers (qPCR)	*SOX2*	GCTACAGCATGATGCAGGACCA/TCTGCGAGCTGGTCATGGAGTT
	OCT4	CCTGAAGCAGAAGAGGATCACC/AAAGCGGCAGATGGTCGTTTGG
	NANOG	CTCCAACATCCTGAACCTCAGC/CGTCACACCATTGCTATTCTTCG
	KLF4	CATCTCAAGGCACACCTGCGAA/TCGGTCGCATTTTTGGCACTGG
House-Keeping Genes (qPCR)	*GAPDH*	AGAAGGCTGGGGCTCATTTG/AGGGGCCATCCACAGTCTTC
Trilineage Markers (qPCR)	*DCX*	TGCCTCAGGGAGTGCGTTA/GAACAGACATAGCTTTCCCCTTC
	IGF2	AGACGTACTGTGCTACCCC/TGCTTCCAGGTGTCATATTGG
	DCN	AGAGTACCTGGTGGGCTGG/GTGGGCAGAAGTCACTTGAT
	AFP	TGAGCACTGTTGCAGAGGAG/TTGTTTGACAGAGTGTCTTGTTGA
	BMP4	GCACTGGTCTTGAGTATCCTG/TGCTGAGGTTAAAGAGGAAACG
Mutation Sequencing	*RP1*	CTCGACAGCAAGCAATAAATTC/GCTCAAGGATGTTAAATACATG
Episomal PCR	Reprogramming episomes	CTTCAACCATCAGGCTTACTTCTA/CTGGTGGGTCAGTAACATCATC

Secondary antibodies were applied for 2 h at room temperature in the dark. Nuclei were stained with DAPI. Controls in which primary antibodies were excluded were performed in parallel. Antibodies and dilutions used are listed in Table 2.

Virtual karyotyping and microsatellite analysis

Virtual karyotyping was performed using the Illumina HumanCoreExome-24 Beadchip SNP array. CNV analysis was performed using GenomeStudio 2.0 (Illumina). Analysis of microsatellite markers was performed using the Promega PowerPlex 16H system (Australian Genome Research Facility).

PCR screening for mycoplasma and episomes

Mycoplasma testing was performed using the Lookout Mycoplasma PCR Detection Kit (Sigma-Aldrich), according to the manufacturer's recommendations. PCR primers for detection of reprogramming episomes are listed in Table 2.

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References

Audo, I., Mohand-Said, S., Dhaenens, C.M., Germain, A., Orhan, E., Antonio, A., Hamel, C., Sahel, J.A., Bhattacharya, S.S., Zeitz, C., 2012. RP1 and autosomal dominant rod-cone dystrophy: novel mutations, a review of published variants, and genotype-phenotype correlation. Hum. Mutat. 33, 73–80.

Ferrari, S., Di Iorio, E., Barbaro, V., Ponzin, D., S. Sorrentino, F., Parmeggiani, F., 2011. Retinitis pigmentosa: genes and disease mechanisms. Curr. Genomics 12, 238–249.

Sullivan, L.S., Heckenlively, J.R., Bowne, S.J., Zuo, J., Hide, W.A., Gal, A., Denton, M., Inglehearn, C.F., Blanton, S.H., Daiger, S.P., 1999. Mutations in a novel retina-specific gene cause autosomal dominant retinitis pigmentosa. Nat. Genet. 22, 255–259.