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Lab Resource: Stem Cell Line

Generation of an induced pluripotent stem cell (iPSC) line from a patient with autosomal dominant retinitis pigmentosa due to a mutation in the NR2E3 gene

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ABSTRACT

A human iPSC line was generated from fibroblasts of a patient affected with autosomal dominant Retinitis Pigmentosa (RP) carrying the mutation p.Gly56Arg in the *NR2E3* gene. The transgene-free iPSCs were generated with the human OSKM transcription factors using the Sendai-virus reprogramming system. iPSCs contained the expected *c.166G>A* substitution in exon 2 of *NR2E3*, expressed the expected pluripotency markers, displayed *in vivo* differentiation potential to the three germ layers and had normal karyotype. This cellular model will provide a powerful tool to study the pathogenesis of *NR2E3*-associated RP.
Resource table.

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Resource utility

This NR2E3-86-iPS cell line constitutes a unique tool to study the pathogenesis of *NR2E3*-associated Retinitis Pigmentosa (RP); essentially by generating photoreceptor cells carrying the mutation in order to determine pathogenic mechanism underlying RP caused by mutation in this photoreceptor-specific gene.

Resource details

NR2E3 (Nuclear Receptor subfamily 2 group E member 3) is a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors, whose expression is restricted to photoreceptors and essential for proper rod and cone photoreceptor differentiation (Mollema and Haider 2010). Most of the disorders linked to mutations in this gene have a recessive mode of inheritance and lead to enhanced S-cone sensitivity syndrome, including Goldmann-Favre syndrome (Schorderet and Escher 2009). Only one mutation (p.Gly56Arg) in the first zinc-finger of the DNA-binding domain of *NR2E3* causes autosomal dominant Retinitis Pigmentosa (RP) with a relatively high prevalence (3 to 3.5%) in Europe (Audo et al. 2012).

In this study, skin fibroblasts from a 49-year-old woman were reprogrammed into iPSCs using non-integrative Sendai viruses containing the reprogramming factors, OCT3/4, SOX2, CMYC, KLF4. The presence of the mutation (*c.166G>A* p.Gly56Arg) in the derived NR2E3-86 iPS cell line was confirmed by Sanger sequencing (Fig. 1A). NR2E3-86 iPS cell colonies displayed a typical ES-like colony morphology and growth behavior and they stained positive for alkaline phosphatase activity (Fig. 1B). We confirmed the clearance of the vectors and the

Unique stem cell line identifier	*IDVi001-A*
Alternative name(s) of stem cell line	*NR2E3-86-iPS*
Institution	Institut de la Vision
Contact information of distributor	Olivier Goureau, olivier.goureau@inserm.fr
Type of cell line	*iPSC*
Origin	*Human*
Additional origin info	*Age: 49-year old* *Sex: female*
Cell Source	*Dermal fibroblasts*
Method of reprogramming	*Transgene free (Sendai Virus)*
Associated disease	*Retinitis Pigmentosa (RP)*
Gene/locus	*NR2E3 (c.166G>A; p.Gly56Arg)*
Method of modification	*N/A*
Gene correction	*NO*
Name of transgene of resistance	*N/A*
Inducible/constitutive system	*N/A*
Date archived/stock date	*Dec. 22, 2016*
Cell line repository/bank	*Not applicable*
Ethical approval	*Approval by French regulatory agencies: CPP Ile de France (2012-A01333-40; P12-02) and the ANSM (B121362-32)*

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exogenous reprogramming factor genes by qPCR after ten passages (Fig. 1C). Immunofluorescence analysis revealed expression of transcription factors OCT4 and NANOG, and surface markers SSEA4 and TRA1-60, characteristics of pluripotent stem cells (Fig. 1D-E). Flow cytometry confirmed the expression of the pluripotent markers SSEA4 and TRA1-81 (Supplementary Fig. 1A). The endogenous expression of the pluripotency associated transcription factors *DNMT3B*, *LIN28A*, *NANOG*, *NODAL*, *POU5F1 (OCT4)*, *TERT* and *GDF3* evaluated by RT-qPCR was

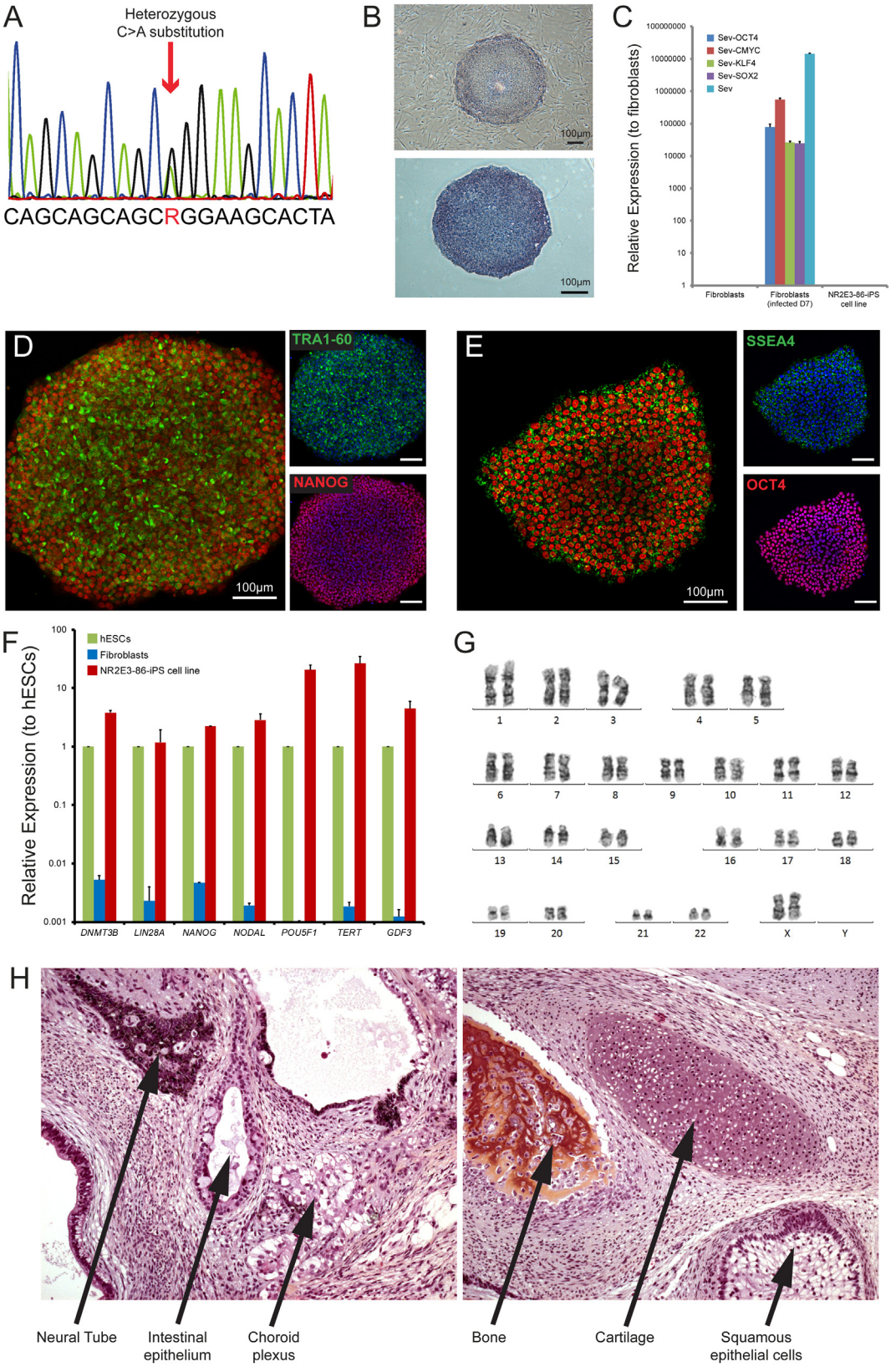

Fig. 1. Characterization of the NR2E3-86-iPS cell line.

comparable with levels observed in human ES cells (hESCs) (Fig. 1F). The NR2E3-86 iPS cell line displayed a normal karyotype (46, XX) after more than twenty culture passages (Fig. 1G). The identity of the iPS cell line with parental fibroblasts and genomic integrity was confirmed by SNP genotyping (Supplementary Fig. 1B). Teratoma assays showed the presence of normal differentiation towards endodermal, ectodermal and mesodermal layers (Fig. 1H). NR2E3-86 iPS cell line was negative for *Mycoplasma* contamination (Supplementary Fig. 1C). Taken together, we have successfully reprogrammed p.Gly56Arg NR2E3 dermal fibroblasts into iPSCs that can be used to generate photoreceptors in order to study pathogenic mechanism underlying RP caused by mutation in the *NR2E3* gene (Table 1).

Materials and methods

Human fibroblast cultures and reprogramming

Small pieces of punch biopsy placed into a T25 cm^2 culture dish were exposed to a minimal amount of media Dulbecco's Modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS), 1 mM sodium pyruvate, Amphotericin β (Fungizone®) and penicillin–streptomycin (all from ThermoFischer Scientific), and incubated at 37 °C in 5% CO_2 in a humidified incubator. The following day, 2 ml of fresh medium was added. When sufficient fibroblast outgrowth had occurred, cells were washed in PBS, dissociated using TrypLE Express (Thermo Fischer Scientific) and re-plated at a split ratio of 1:3. Human fibroblasts at 50–80% of confluence were transduced using the CytoTune Sendai reprogramming vectors Oct4, Klf4, Sox2 and c-Myc (ThermoFischer Scientific) and cultured for 6 days in fibroblast medium before to be plate on mitomycined human foreskin (MHF)-seeded dishes. The day after, the fibroblast medium was replaced with the iPS medium, corresponding to ReproStem medium (ReproCELL, Ozyme) supplemented with 10 ng/ml of human recombinant FGF2 (PreproTech France). The emergent hiPSC colonies were picked under a stereomicroscope according to their hESC-like colony morphology and expanded on feeder layers. After generation of a frozen stocks, hiPSCs were cultured on MHF feeder layers or preferentially adapted and cultured in feeder free conditions (Reichman et al. 2017) on truncated recombinant human vitronectin-coated dishes with Essential 8™ medium (both from ThermoFischer Scientific). Absence of mycoplasma contamination was verified by the MycoAlert™ Mycoplasma Detection Kit (selective biochemical test of mycoplasma enzymes) according to the manufacturer's instructions (Lonza).

After ten passages, the clearance of the exogenous reprogramming factors and Sendai virus genome was confirmed by qPCR following the manufacturer's instructions (ThermoFischer Scientific) (Table 2).

Mutation analysis

Genomic DNA from hiPSCs was extracted with Nucleospin Tissue Kit (Macherey-Nagel) according to the manufacturer instruction. PCR amplification flanking exon 2 of *NR2E3* gene (Table 2) was performed using HOT FIRE Pol DNA Polymerase (Solis BioDyne). PCR products were sequenced using BigDye® Terminator v1.1 Cycle Sequencing Kit (ThermoFischer Scientific) on a 3730 DNA analyzer (Applied Biosystems).

Karyotype analyses

Conventional cytogenetic analysis was performed as described previously (Reichman et al. 2014). Molecular karyotype was analyzed by SNP genotyping using Illumina's Infinium HumanCore-24 Bead Chips (Illumina, Inc., San Diego, USA) at Integragen (Evry, France). Processing was performed on genomic DNA following the manufacturer's procedures. LogR ratio and B allele plots were generated in GenomeStudio software (Illumina, Inc.).

In vivo pluripotency analysis by teratoma formation assay

Teratoma assays were performed as described previously (Reichman et al. 2014).

Real-Time PCR analysis

Total RNAs were extracted using Nucleospin RNA II kit (Macherey-Nagel) and cDNA synthesized using the QuantiTect reverse transcription kit (Qiagen) following manufacturer's recommendations. qPCR analysis was performed in three minimum independent biological experiments with custom TaqMan® Array 96-Well Fast plates (Thermo Fischer Scientific) according to the manufacturer's protocol as described previously (Reichman et al. 2014) (Table 2) .

Flow cytometry

Cells were detached with Accutase solution and harvested for quantitative analysis by flow cytometry (Cytomics FC500 MCL; Beckman Coulter) by staining the TRA-1-81, and SSEA-4 antibodies (Table 2) and data were analyzed with FlowJo software.

Alkaline phosphatase and immunofluorescence staining

Staining of fixed hiPSCs was performed as described previously (Reichman et al. 2014) (Table 2).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	*hESC-like morphology*	Fig. 1, panel B
	AP staining	*Positive*	
Phenotype	qPCR	*Expression of pluripotency markers: DNMT3B, LIN28A, NANOG, NODAL, POU5F1, TERT, GDF3*	Fig. 1, panel F
	Immunohistochemistry	*Expression of pluripotency markers: OCT4, NANOG, SSEA4 and TRA1–60*	Fig. 1, panels D and E
	Flow cytometry	*SSEA-4:97.8% and TRA1-81: 95.1%*	Supplementary Fig. 1A
Genotype	Karyotype (G-banding) and resolution	*46XX,* *Resolution 450–500*	Fig. 1, panel G
	SNP array analysis	*Genomic integrity*	Supplementary Fig. 1B
Identity	SNP array analysis	*Genomic integrity and identity (parental fibroblasts and the respective iPS cell line)*	Supplementary Fig. 1B
Mutation analysis (IF APPLICABLE)	Sequencing	*Heterozygous (G>A)*	Fig. 1, panel A
Microbiology and virology	Mycoplasma	*Mycoplasma testing by luminescence: Negative*	Supplementary Fig. 1C
Differentiation potential	Teratoma formation	*Representation of all three germ layers formation*	Fig. 1, panel H
Donor screening (OPTIONAL)	N/A		
Genotype additional (OPTIONAL)	N/A		

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow cytometry			
	Antibody	Dilution	Company Cat # and RRID
OCT4	Rabbit mAb anti-OCT4	1:300	Cell Signaling Technology Cat#30A3, RRID:AB_2840
NANOG	Rabbit mAb anti-NANOG	1:300	Cell Signaling Technology Cat#D73G4, RRID:AB_4903
SSEA4	Mouse mAb anti-SSEA4	1:300	Cell Signaling Technology Cat#MC813, RRID:AB_4755
TRA1–60	Mouse mAb anti-TRA1–60(S)	1:300	Cell Signaling Technology Cat#TRA-1-60(S), RRID:AB_4746
TRA1–81 PE- conjugated	Mouse IgM anti Human TRA-1-81	1:50	R&D Systems Cat#TRA-1-81, RRID:AB_FAB8495P-025
SSEA4 APC-conjugated	Mouse IgG_3 anti Human/Mouse SSEA-4	1:50	R&D Systems Cat#MC-813-70, RRID:AB_FAB1435A-025
Secondary antibodies	Alexa Fluor 594-conjugated Donkey anti-Rabbit IgG (H + L),	1:600	Thermo Fisher Scientific Cat# A-21207, RRID: AB_141637
	Alexa Fluor 488-conjugated Donkey anti-Mouse IgG (H + L)	1:600	Thermo Fisher Scientific Cat# A-21202, RRID: AB_141607

Primers		
	Target	Forward/Reverse primer (5′-3′)
Targeted mutation sequencing	NR2E3 Exons2-3P4	GTGTGGATGCACAGTGAGG/CTGTTATAAGGCTGGCCATG
Elimination of Sendai virus transgenes (qPCR - TaqMan)	*SeV*	Assay ID: Mr04269880_mr
	SeV-OCT4	Assay ID: Mr04269878_mr
	Sev-KLF4	Assay ID: Mr04269879_mr
	Sev-SOX2	Assay ID: Mr04269881_mr
	Sev-CMYC	Assay ID: Mr04269876_mr
Pluripotency markers (qPCR - TaqMan)	*DNMT3B*	Assay ID: DNMT3B-Hs00171876_m1
	GDF3	Assay ID: GDF3-Hs00220998_m1
	LIN28	Assay ID: LIN28A-Hs00702808_s1
	NANOG	Assay ID: NANOG-Hs02387400_g1
	NODAL	Assay ID: NODAL-Hs00415443_m1
	POU5F1 (OCT4)	Assay ID: POU5F1-Hs00999632_g1
	TERT	Assay ID: TERT-Hs00972656_m1
House-keeping genes (qPCR - TaqMan)	*18S*	Assay ID: 18S-Hs99999901_s1

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2017.08.003.

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Author disclosure statement

There are no competing financial interests in this study.

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