Stem Cell Research 27 (2018) 86–89

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Stem Cell Line

Generation of induced pluripotent stem cell line, CSSi002-A (2851), from a patient with juvenile Huntington Disease

Jessica Rosati [a,*], Eris Bidollari [a], Giovannina Rotundo [a], Daniela Ferrari [b], Barbara Torres [c], Laura Bernardini [c], Federica Consoli [d], Alessandro De Luca [d], Iolanda Santimone [e], Giuseppe Lamorte [d], Ferdinando Squitieri [e], Angelo Luigi Vescovi [a]

[a] IRCCS Casa Sollievo della Sofferenza, Cellular Reprogramming Unit, Viale dei Cappuccini, San Giovanni Rotondo, Foggia 71013, Italy
[b] Biotechnology and Bioscience Department Bicocca University, Piazza della Scienza 2, Milan 20126, Italy
[c] IRCCS Casa Sollievo della Sofferenza, Cytogenetic Unit, Viale dei Cappuccini, San Giovanni Rotondo, Foggia 71013, Italy
[d] IRCCS Casa Sollievo della Sofferenza, Molecular Genetics Unit, Viale dei Cappuccini, San Giovanni Rotondo, Foggia 71013, Italy
[e] IRCCS Casa Sollievo della Sofferenza, Huntington and Rare Disease Unit, Viale dei Cappuccini, San Giovanni Rotondo, Foggia 71013, Italy

ARTICLE INFO

Article history:
Received 12 December 2017
Received in revised form 29 December 2017
Accepted 8 January 2018
Available online 9 January 2018

ABSTRACT

Huntington Disease (HD) is an autosomal dominant disorder characterized by motor, cognitive and behavioral features caused by a CAG expansion in the *HTT* gene beyond 35 repeats. The juvenile form (JHD) may begin before the age of 20 years and is associated with expanded alleles as long as 60 or more CAG repeats. In this study, induced pluripotent stem cells were generated from skin fibroblasts of a 8-year-old child carrying a large size mutation of 84 CAG repeats in the *HTT* gene. HD appeared at age 3 with mixed psychiatric (i.e. autistic spectrum disorder) and motor (i.e. dystonia) manifestations.

Resource table

Unique stem cell line identifier	CSSi002-A (2851)
Alternative name(s) of stem cell line	HD 8 yrs
Institution	Cellular Reprogramming Unit, IRCCS Casa Sollievo della Sofferenza, Viale dei Cappuccini, San Giovanni Rotondo, Foggia Italy
Contact information of distributor	Jessica Rosati j.rosati@css-mendel.it
Type of cell line	iPSC
Origin	human
Additional origin info	Age: 8 Sex: F Ethnicity: Caucasian
Cell Source	Skin Fibroblasts
Clonality	Clonal
Method of reprogramming	Non integrating episomal vectors
Genetic modification	NO
Type of modification	N/A
Associated disease	Juvenile Huntington Disease
Gene/locus	IT15/HD GENE- 4p16.3
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	May 2017
Cell line repository/bank	N/A
Ethical approval	Casa Sollievo della Sofferenza Ethical Committee, approval number: 75/CE

Resource utility

This iPSC line offers an useful resource for investigating pathogenic mechanisms in Juvenile Huntington Disease during neural differentiation, as well as for discovering novel drugs capable of reducing or reverting pathogenic mechanisms.

Resource details

Although the onset of pathological traits in most HD patients is in the adult age, a small but significant proportion presents in pediatric patients. The clinical presentation of JHD is generally different from adult HD and is, in most cases, characterized by features of parkinsonism. The course of childhood-onset Huntington Disease is a rapid downhill course. The disease progresses more rapidly in children than in adults. Three phases of childhood-onset HD have been described: (1) an initial phase of behavioral disorder, learning difficulty, gait disturbance, and mild chorea; (2) a phase with signs of mental deterioration, rigidity, speech disturbance, and seizures; and (3) a terminal phase of bed confinement, hypotonia, and increasing seizures. Juvenile Huntington

* Corresponding author.
E-mail address: j.rosati@css-mendel.it (J. Rosati).

https://doi.org/10.1016/j.scr.2018.01.011

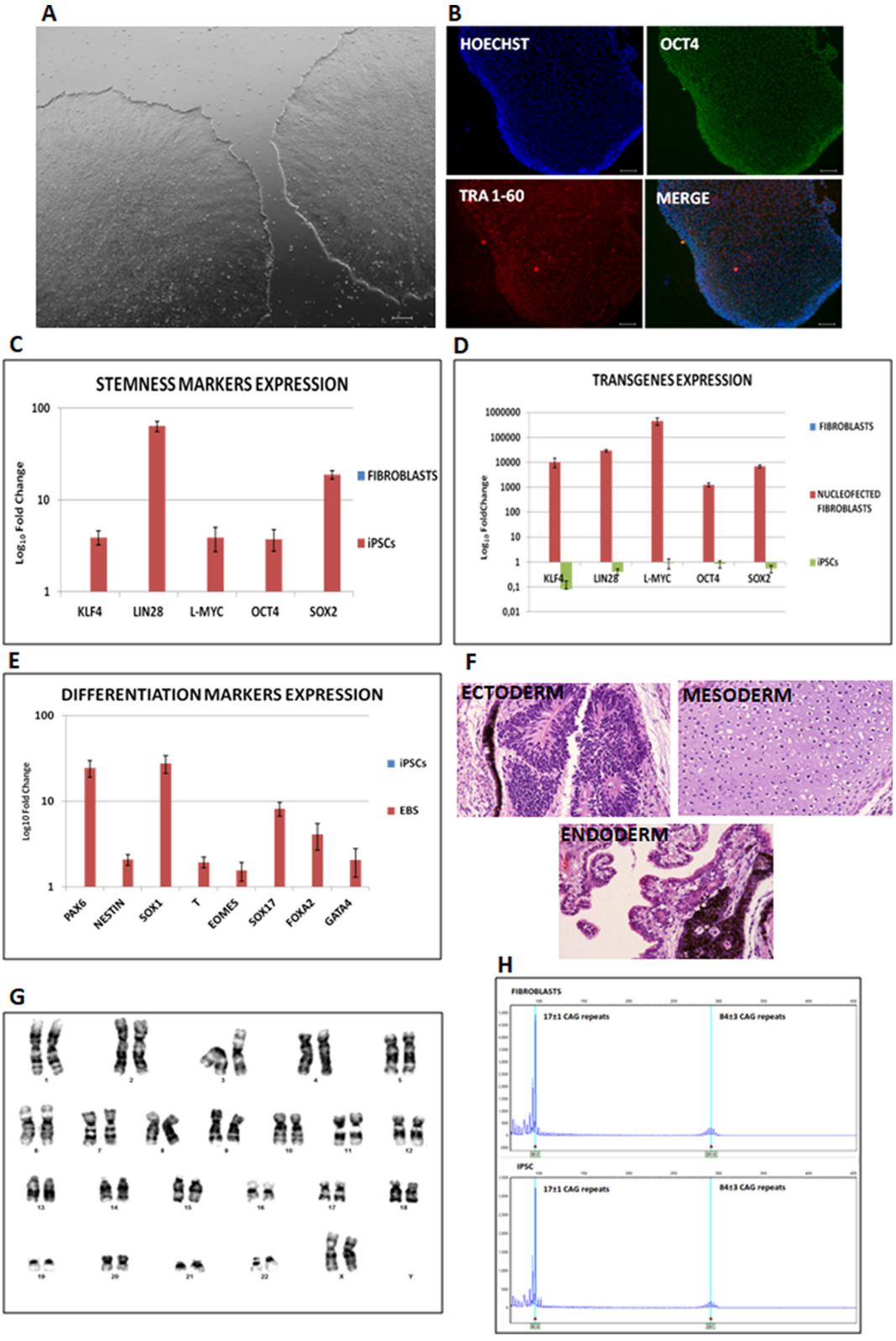

Fig. 1. Production and characterization of CSSi002A (2851) iPSC. A. iPSC phase contrast image, scale bars correspond to 100 μm. B. Immunofluorescence analysis of pluripotency markers OCT4 and TRA1-60 in iPSC, scale bars correspond to 100 μm. C. qPCR analysis of pluripotency markers; histograms show the expression of endogenous transcripts: KLF4, LIN28, L-MYC, OCT4, SOX2 in iPSC, fibroblasts and 18S are used as references. D. qPCR analysis of pluripotency transgenic markers. E. qPCR analysis of differentiation markers in iPSC and in embryoid bodies, iPSC and 18S are used as references. F. Histological analysis of teratoma: derivatives of all three germ layers. G. Representative karyotype of iPSC. H. PCR results show 17 and 83 CAG repeats in fibroblasts and 17 and 83 CAG repeats in IPS cell line.

Disease (JHD) subjects are excluded from therapeutic trials due to missing biomarkers, validated evaluation scales and their atypical, yet unpredictable, presentation (Quarrell et al., 2013). Following institutional ethical committee approval and patient informed consent, dermal fibroblasts were isolated by explant culture of a skin biopsy obtained from 8-year-old child. Patient fibroblasts were reprogrammed to iPSCs by

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal morphology	Fig. 1A
Phenotype	Immunocytochemistry	Staining of pluripotency markers: Oct4; Tra-1-60	Fig. 1B
	qPCR	Expression of endogenous stemness markers: OCT4, LIN28, L-MYC, KLF4, SOX2 Silenced transgene expression (from episomes): OCT4, LIN28, L-MYC, KLF4, SOX2	Fig. 1C, D
Genotype	Karyotype (G-banding) and resolution	46 XX, resolution: 450–500 band level	Fig. 1G
Identity	STR analysis	Tested 19 sites: all matched	Data is available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	PCR results with 17 ± 1 and 84 ± 3 CAG repeats in fibroblast and 17 ± 1 and 84 ± 3 CAG repeats in IPS cell line.	Fig. 1H
Microbiology and virology	Mycoplasma	Mycoplasma tested by EZ-PCR: negative	Supplementary Fig. 1
Differentiation potential	Embryoid body formation and Teratoma formation	Genes expressed in embryod bodies: SOX1, NESTIN, PAX6, EOMES, T, GATA4, FOXA2, SOX17 Teratoma: proof of three germ layers formation.	Fig. 1E, F

nucleofection with three episomal plasmids encoding human L-MYC and LIN28, SOX2 and KLF4, and OCT4 combined with a short hairpin RNA for P53 (shP53) (Okita et al., 2011). The iPSC line described in this publication was termed CSSi002-A (2851). iPS cell colonies displayed a typical Embryonic Stem cell-like colony morphology (Fig. 1A) and stained positive for OCT4 and TRA-1-60 (Fig. 1B). The expression of pluripotency genes *OCT4*, L-*MYC*, *KLF4*, *SOX2* and *LIN28* was higher in iPSCs compared to patient fibroblasts (Fig. 1C) while exogenous gene expression (from episomes) was silenced during amplification, as demonstrated by qPCR performed with primers against exogenous genes; as positive control, human fibroblasts 72 h post nucleofection were used (Fig. 1D). Pluripotency was supported by the capability of CSSi002-A (2851) to differentiate into three germ layers in vitro, as confirmed by qPCR showing expression of the endodermal markers SOX17, FOXA2 and GATA4, the mesodermal markers Brachyury (T) and EOMES and the ectodermal markers SOX1 and NESTIN (Fig. 1E). Differentiation capacity into three germ layers was also demonstrated by in vivo teratoma formation (Fig. 1F). iPSC had a numerically and structurally normal karyotype (46, XX) (Fig. 1G). iPSC were negative for Mycoplasma contamination (Supplementary Fig. 1). STR analysis showed that parental fibroblasts and the newly created iPSC lines shared alleles with a 100% match (STR data is available with the authors).

Materials and methods

Skin biopsy and fibroblast reprogramming

The skin biopsy was dissected and cultured in fibroblast medium (DMEM high glucose, 20% FBS, 2 mM L-glutamine and 1% penicillin–streptomycin (all reagents from Sigma Aldrich)) for 30 days at 37 °C and 5% CO2 to allow fibroblasts to grow out. Briefly, 1×10^5 fibroblasts were nucleofected with 3 μg 1:1:1 mix of the episomal plasmids pCXLE-hUL (Addgene #27080), pCXLE-hSK (Addgene #27078) and pCXLE-hOCT4-shp53 (Addgene #27077). The nucleofected cells were plated in fibroblast medium for one week. On day 7 the cells were counted and plated on Matrigel (1100) (BD Biosciences). From day 8 the cells were cultured in NutristemXF medium (Biological Industries). When iPSC colonies reached a sufficient size, they were manually cut and passaged for expansion. Absence of mycoplasma contamination was verified by PCR analysis using EZ-PCR kit (Biological Industries).

Immunofluorescence staining

Cells were fixed using 4% paraformaldehyde for 20 min at room temperature. The cells were incubated with Blocking Buffer (PBS, 20% Normal Goat Serum, 0.1% Triton X-100) for 30 min at room temperature. Next, primary antibodies diluted in blocking buffer were incubated overnight at 4 °C. After washing, Alexa Fluor 594- and/or Alexa Fluor 488-conjugated secondary antibodies in PBS 1x were added 1 h at room temperature. Nuclei were counterstained with DAPI. Micrographs were taken using a Nikon C2 fluorescence microscope and NIS Elements 1.49 software (Table 1).

Table 2
Reagents details.

Antibodies used for immunocytochemistry

	Antibody	Dilution	Company cat # and RRID
Pluripotency markers	Rabbit anti-OCT4	1:100	Life technologies (A13998)
	Mouse anti-TRA-1-60	1:100	Life technologies (411000)
Secondary antibodies	Anti-rabbit AlexaFluor 488	1:10000	Invitrogen (A11034)
	Anti-mouse AlexaFluor 555	1:10000	Invitrogen(A21422)

Primers

	Target	Forward/reverse primer (5′-3′)
Episomal genes	eOCT4	Fwd: CAT TCA AAC TGA GGT AAG GG
		Rev: TAG CGT AAA AGG AGC AAC ATA G
	eKLF4	Fwd: CCA CCT CGC CTT ACA CAT GAA GA
		Rev: TAG CGT AAA AGG AGC AAC ATA G
	eLIN28	Fwd: AGC CAT ATG GTA GCC TCA TGT CCG C
		Rev: TAG CGT AAA AGG AGC AAC ATA G
	eL-MYC	Fwd: GGC TGA GAA GAG GAT GGC TAC
		Rev: TTT GTT TGA CAG GAG CGA CAA T
	eSOX2	Fwd: TTC ACA TGT CCC AGC ACT ACC AGA
		Rev: TTT GTT TGA CAG GAG CGA CAA T
Pluripotency genes	OCT4	Fwd: CCC CAG GGC CCC ATT TTG GTA CC
		Rev: ACC TCA GTT TGA ATG CAT GGG AGA GC
	KLF4	Fwd: ACC CAT CCT TCC TGC CCG ATC AGA
		Rev: TTG GTA ATG GAG CGG CGG GAC TTG
	LIN28	Fwd: AGC CAT ATG GTA GCC TCA TGT CCG C
		Rev: TCA ATT CTG TGC CTC CGG GAG CAG GGT AGG
	L-MYC	Fwd: GCG AAC CCA AGA CCC AGG CCT GCT CC
		Rev: CAG GGG GTC TGC TCG CAC CGT GAT G
	SOX2	Fwd: TTC ACA TGT CCC AGC ACT ACC AGA
		Rev: TCA CAT GTG TGA GAG GGG CAG TGT GC
	18S	Fwd: GGC CCT GTA ATT GGA ATG AGT C
		Rev: CCA AGA TCC AAC TAC GAG CTT
Differentiation markers	SOX1	Hs01057642_s1
	NESTIN	Hs04187831_g1
	PAX6	Hs00240871_m1
	EOMES	Hs00172872_m1
	T	Hs00610080_m1
	GATA4	Hs00171403_m1
	FOXA2	Hs00232764_m1
	SOX17	Hs01057642_s1
	18S	Hs03003631_g1

Real-time PCR analysis

Total RNA was isolated from cultured cells with Trizol (Life Technologies). Reverse transcription of 1 μg of RNA was performed using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) after digestion with DNAse I (Life Technologies). Each RNA sample was tested in duplicate and 18S was used to normalize transcript abundance and calculations were performed with the delta Ct method. Statistical analyses were performed on three independent experiments. Primers are listed in Table 2.

In vitro spontaneous differentiation

The cell clumps from iPS were plated on Petri dishes in Nutristem medium. One day later, Nutristem medium was substituted with in differentiation medium: DMEM/F12, 20% KSR (Gibco), 0,1 mM NEAA, 0.1 mM β-mercaptoethanol, 1% Pen/Strep. The embryoid bodies (EBs) were grown for two weeks.

Karyotyping

iPSCs were cultured in chamber slides (Thermo Fisher Scientific) coated with Matrigel in NutristemXF medium for 2–3 days. Cells were treated with a 0.1 μg/mL COLCEMID solution (Thermo Fisher Scientific) for 60 min at 37 °C. Metaphases were obtained by adding 30 mM KCl in 10%FBS at 37 °C for 6 min and by fixation using cold fresh-made 3:1 ethanol:acetic acid solution. Karyotype analysis was carried out on GTG-banded metaphases. Fifteen metaphases were counted and three karyotypes analyzed. Only clonal aberration were considered, following the ISCN recommendations.

STR analysis

Fibroblasts and iPSCs DNA was extracted by Dneasy blood and tissue kit (QIAGEN). PCR amplification of 19 distinct STRs (D13S252, D13S305, D13S634, D13S800, D13S628, D18S819, D18S535, D18S978, D18S386, D18S390, D21S11, D21S1437, D21S1409, D21S1442, D21S1435, D21S1446, DXS6803, XHPRT, DXS1187) was carried out using the QST*Rplusv2 kit (Elucigene Diagnostics), PCR products were separated on an ABI Prism 3130 DNA sequencer and analyzed by GeneMapper version 4.0 (Applied Biosystems).

Teratoma formation

Approximately 3×10^6 dispase-treated iPSCs, in 100 μl of Matrigel, were injected into the right flank of nude mice, following ethical guidelines. About 4–6 weeks after injection, tumors were dissected, fixed in 10% formalin (Sigma Aldrich), paraffin-embedded, sectioned and stained with hematoxylin/eosin. The presence of differentiated tissues representative of the three embryonic germ layers was analyzed.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.01.011.

Acknowledgment

This work was supported by Italian Ministry of Health, Ricerca corrente 2017 to JR, Ricerca corrente 2017 to FS, Ricerca Finalizzata RF-2016-02364123 to FS.

References

Okita, K., Matsumura, Y., Sato, Y., Okada, A., ... Yamanaka, S., 2011. A more efficient method to generate integration-free human iPS cells. Nat. Methods 8 (5), 409–412.

Quarrell, O.W.J., Nance, M.A., Nopoulos, P., ... Squitieri, F., 2013. Managing juvenile Huntington's disease. Neurodegener. Dis. Manag. 3 (3). https://doi.org/10.2217/nmt.13.18.