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Lab Resource: Single Cell Lines

Induced pluripotent stem cell line (INSAi001-A) from a Gaucher disease type 3 patient compound heterozygote for mutations in the GBA1 gene

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ABSTRACT

Gaucher Disease (GD) type 3 is a neurological form of a multisystemic autosomal recessive disorder belonging to the group of lysosomal storage diseases. Causal mutations in the glucocerebrosidase 1 (GBA1) commonly lead to abnormal protein and GD, heterozygosity is a genetic risk factor for Parkinson's disease. This work describes the use of a non-integrative approach using Sendai Virus delivery to establish induced Pluripotent Stem Cells (iPSCs) from fibroblasts from a GD type 3 patient. Differentiation of iPSCs can be employed to generate a variety of complex cell types with a high degree of genetic complexity that would otherwise be unattainable.

Resource table

Unique stem cell line identifier	INSAi001-A
Alternative name(s) of stem cell line	GD3-1
Institution	INSA
Contact information of distributor	Olga Amaral
Type of cell line	Induced pluripotent stem cell line (iPSC)
Origin	Human (Istituto "Giannina Gaslini" Biobank)
Additional origin info	Age: 27 years Sex: male Ethnicity if known: unknown
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	CytoTune™-iPS 2.0 Sendai Reprogramming Kit
Genetic Modification	Yes
Type of Modification	Familial
Associated disease	Gaucher Disease
Gene/locus	GBA1 gene (Gene ID: 2629)/locus 1q22; Mutations in compound heterozygosity: p.L444P (rs421016) and p.F213I (rs381737)
Method of modification	Not applicable
Name of transgene or resistance	Not applicable
Inducible/constitutive system	Not applicable
Date archived/stock date	2019/06/25
Cell line repository/bank	The cell line has not been deposited in a stem cell bank or repository but is physically cryopreserved at INSA's facilities in CSPGF in Porto, Portugal.
Ethical approval	Original fibroblast line was obtained from a non-commercial provider, Gaslini Institute Biobank under Genetic Biobank Guidelines, Telethon - SIGU, Analysis 4/5, 2003. Italian Data Protection Authority (Garante Privacy), General Authorisation for the processing of genetic data no. 8/2016.

1. Resource utility

Lysosomal Storage Diseases (LSDs) may affect hard to reach cells, such as cardiac cells and neurons, which require invasive procedures to access the disease-target-tissue. The present work constitutes a first stage towards the final goal of differentiating iPSCs to generate specific

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cell types to aid model pathogenesis and testing of potential therapeutics. It represents a useful tool for optimization of therapeutic protocols and identification of key factors regulating pathways in organ involvement.

2. Resource details

This work focuses on the development of iPSCs from fibroblasts using non-integrative approaches in a cell line of one of the most common sphingolipidoses, Gaucher disease (GD). GD is a LSD usually caused by mutations in the glucocerebrosidase 1 (GBA1, gene ID:2629), which lead to defective glucocerebrosidase (GlcCerase, EC 3.2.1.45). Clinically, GD encompasses a continuum of clinical findings with multisystemic involvement, which for diagnosis purposes is divided in three main categories. This work used type 3 GD fibroblasts (ORPHA:77,261, OMIM:231,000). GD type 3, with late onset neurological involvement, shares clinical features of type 1 and type 2. Over the years, genetic analyses of Parkinson Disease (PD) patients have demonstrated that GBA1 missense mutations constitute a relatively common risk factor for developing PD. The continuum of clinical symptoms of GD now encompasses a wide variety of neurologic symptoms in addition to the multisystemic features.

The original manuscript regarding this cell line had a concomitant report of two patients with similar GBA1 genotypes but different clinical forms. In such cases, it is particularly difficult to evaluate how modifying factors can contribute to the different clinical disease expression. The patient whose cells were used for generating INSAi001-A was classified as type 3, while a younger patient, with similar genotype, was type 1 [Filocamo et al., 2002]. Such divergences in symptoms and classifications make the use of iPSCs particular interesting for modelling and pathogenesis studies. As stated in the original paper, the type 3 patient was diagnosed at age five and splenectomyzed at age ten, regardless of enzyme therapy he later developed untreatable epilepsy [Filocamo et al., 2002]. Interestingly, both mutations exist as pseudogene sequence and they both exist in patients of Japanese origin Ida et al., 1997.

Differentiation of iPSCs, INSAi001-A, can generate a variety of complex cell types with a high degree of genetic complexity that would otherwise be unattainable and might help understand the pathogenesis of GD in such cases. An iPS cell line, INSAi001-A, was successfully generated from skin fibroblasts from a compound heterozygous patient.

All results are summarized in Fig. 1. The fibroblasts were reprogrammed by co-expressing Yamanaka's factors, OCT3/4, SOX2, KLF4, and cMYC through the integration-free Sendai virus gene-delivery method on a feeder-free system with vitronectin (Fig. 1A). The INSAi001-A cell line, showed endogenous expression of the pluripotency markers by positive immunofluorescence staining for NANOG, SOX2, OCT4 and TRA-1–60 (Fig. 1B1-4) further quantitative analysis also showed elevated OCT4 by RT-qPCR (Fig. 1C). The resulting iPSCs INSAi001-A had a normal karyotype (Fig. 1D) and retained the GD-causing GBA1 mutations (Fig. 1E). Differentiation potential can be seen in Fig. 1F where formation potential of the three germ layers is shown by positive Immunofluorescence staining for mesoderm (Brachyury), endoderm (SOX17) and ectoderm (Otx2). The use of Sendai Virus (SeV) vectors under feeder free conditions proved to be effective in producing iPSCs that maintain the original genetic characteristics of the donor fibroblasts, as far as the extent of the examination as shown by the causal mutations, gene and STR analysis.

3. Materials and methods

3.1. Biological sample

The skin fibroblast cell line from GD-3 patient was obtained from Instituto Giannina Gaslini Bioresource Bank in Geneva, Italy in compliance with the ethical recommendations issued by the Oviedo agreement of 1997 and the International Declaration on Human Genetic Data of 2003 Filocamo et al., 2014. The GD cell line used was from a type 3 patient who bears a compound heterozygous genotype with p.L444P (rs421016) and p.F213I (rs381737).

3.2. Fibroblast culture

Cells were maintained in DMEM(1x) + GlutaMAX™ medium (Gibco™) supplemented with 10% foetal bovine serum in a cell incubator.

3.3. Reprogramming of fibroblasts to iPSCs

Cellular reprograming of fibroblasts to iPSCs was carried out using a non-integrative approach. SeV reprogramming was carried out successfully on skin fibroblasts using CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Invitrogen™). As reprogramming matrix Vitronectin (VTN−N from Gibco™) was used. By the 10th passage SeV was no longer detected by PCR indicating that the experiment had been successful and the iPSCs were free of viral footprint. Characterisation and validation is shown in Fig. 1 and Table 1.

3.4. Cell culture and passage

Cells were cultured on 60 mm plates coated with 1/100 Vitronectin (VTN−N) Recombinant Human Protein Truncated diluted in 1X DPBS. Incubation was at 37°C with 5% CO2 in a Refurbished Binder CB 150 Air-Jacketed CO2 Incubator. Once high confluency is reached, the medium is removed, cells are washed with 1 mL of 1X DPBS and treated with 1 mL of 0,5 mM EDTA solution, after 4 min of incubation at room temperature, EDTA is removed. Cell passage is carried out by resuspension in Gibco™ StemFlex™ Medium with 1% Lonza BioWhittaker™ PEN-STREP 5000 U Penicilin/mL and 10% Gibco™ StemFlex™ Suplement (10X); 50 µL of cell suspension are added to new matrix treated dishes in a 2 mL volume of medium.

3.5. Pluripotency characterisation

As determined by real-time quantitative analysis (Fig. 1C), expression of the pluripotency gene OCT4 was upregulated in iPSCs compared to the patient fibroblasts. As seen in Fig. 1B.1–4 qualitative analysis by immunofluorescence with NANOG, SOX2, OCT4 and TRA-1–60 antibodies (indicated in Table 2), showed positive staining for all pluripotency markers confirming pluripotent status.

3.6. Genetic characterisation

Genotype was established in fibroblasts and in iPSCs and the mutations confirmed (Fig. 1E). See Table 2 for primers used. PCR products were purified with ExoSAP™-IT (Applied Biosystems™), for Sanger sequencing BigDye®Terminator v3.1 (Applied Biosystems™) was used. STR analysis used the AmpFeSTR® Identifier® (Applied Biosystems™).

Karyotyping was performed on G-banded metaphase chromosomes using standard procedures and at least five metaphases were examined per sample. As seen in Fig. 1D, the Karyotype was normal (46, XY). Quantitative real time polymerase chain reaction (qRT-PCR) was carried out on RNA isolated using TRIzol Reagent (Ambion®). 0.5 µg RNA/reaction was reverse transcribed using qScript cDNA supermix (Quanta BIO). Real Time RT-PCR was performed using TaqMan™ Universal PCR mastermix (Applied Biosystems™). CT-values were normalized to the housekeeping gene GAPDH using the ΔΔCT-method. Primers are listed in Table 2.

3.7. Fluorescent immunocytochemistry

To confirm true pluripotency capability of the derived iPSCs, a three

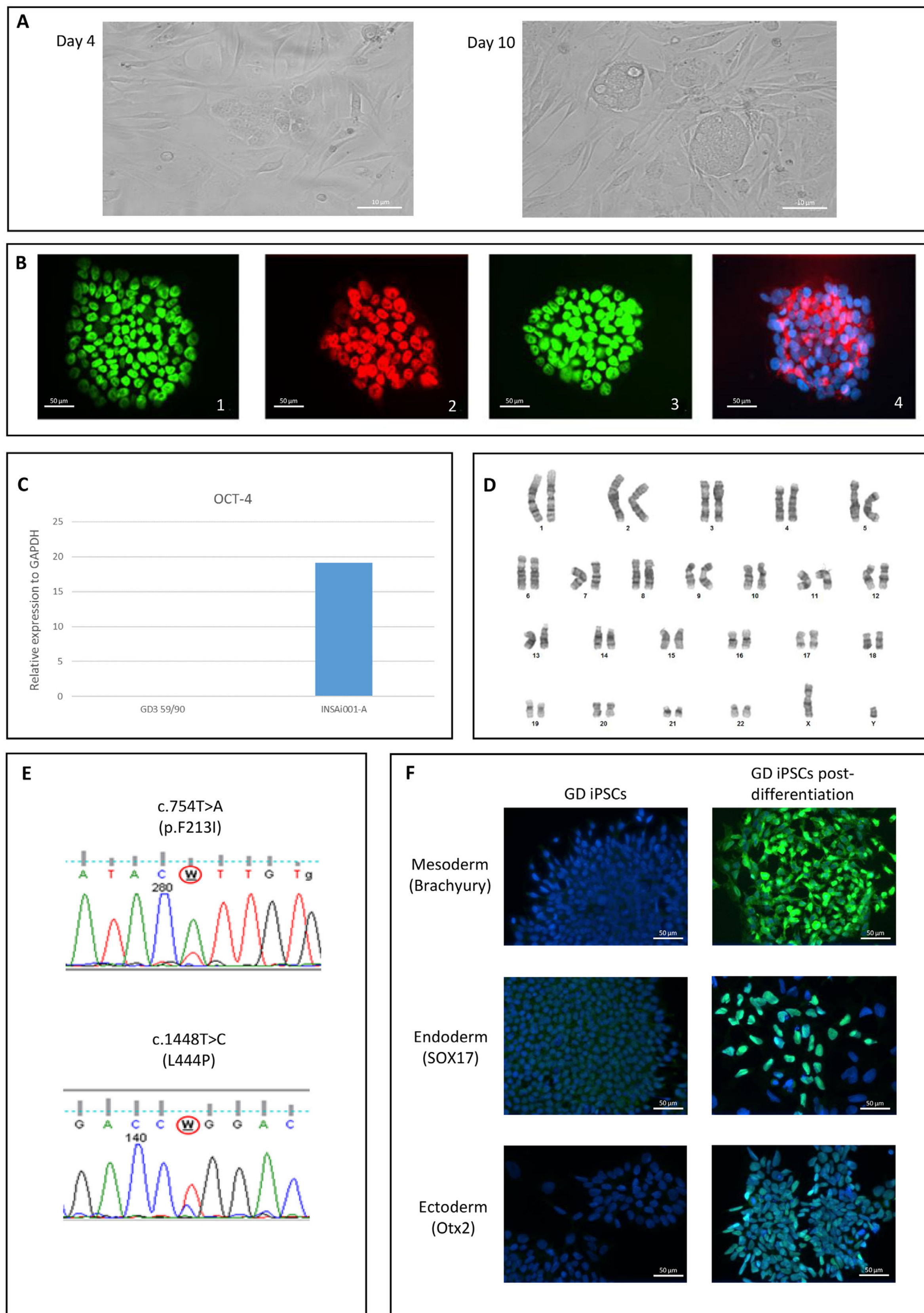

Fig. 1. Characterisation of induced pluripotent stem cell line (INSAi001-A) from A Gaucher type 3 patient.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography (clear field microscopy)	Visual inspection was normal	Fig. 1 panel A
Phenotype	Qualitative analysis (Immunofluorescence)	Positive for NANOG, SOX2, OCT4 and TRA–1–60 (with DAPI)	Fig. 1 panel B
	Quantitative analysis (RT-qPCR)	Expression of OCT4	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46XY, Resolution 450–500	Fig. 1 panel D
Identity	STR analysis	DNA Profiling Performed	Supplementary file
Mutation analysis	Sequencing	Compound heterozygous: p.L444P (rs421016) and p.F213I (rs381737)	Fig. 1 panel E
Microbiology and virology	PanRac AppliChem PCR Mycoplasma Test Kit (A3744)	Mycoplasma testing: Negative	Not shown but available with author
Differentiation potential	Directed differentiation (Immunofluorescence) and embryoid body formation	Formation of three germ layers: positive for Otx2 (ectoderm marker), Brachyury (mesoderm marker), and SOX17 (endoderm marker)	Fig. 1 panel F

Table 2
Reagent details.

Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Mouse anti-Nanog, clone 7F7.1, Alexa Fluor® 488 conjugate	1:100	Millipore Cat# MABD24, RRID:AB_11,203,826
	Mouse anti-Sox-2, clone 10H9.1, Cy3 conjugate	1:100	Millipore Cat# MAB4423, RRID:AB_11,205,572
	Mouse anti-Oct-4 (POU5f1), clone 7F9.2, Alexa Fluor® 488 conjugate	1:100	Millipore Cat# MAB4419, RRID:AB_1,977,399
	Mouse anti-TRA–1–60, clone TRA–1–60, Cy3 conjugate	1:100	Millipore Cat# MAB4360, RRID:AB_2,119,183
Germ layer markers	Goat Anti-Human Brachyury Polyclonal Antibody, unconjugated (Mesoderm)	1:10	R and D Systems Cat# AF2085, RRID:AB_2,200,235
	Goat Anti-Human Sox17 Polyclonal Antibody, unconjugated (Endoderm)	1:10	R and D Systems Cat# AF1924, RRID:AB_355,060
	Goat Anti-Human Otx2 Polyclonal Antibody, unconjugated (Ectoderm)	1:10	R and D Systems Cat# AF1979, RRID:AB_2,157,172
Secondary antibodies	Donkey anti-Goat IgG ($H + +L$) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:200	Thermo Fisher Scientific Cat# A-11,055, RRID:AB_2,534,102
Primers			
	Target	TaqMan® assay	
Pluripotency marker (qPCR)	OCT-4	hs00999632_g1	
House-keeping gene (qPCR)	GAPDH	hs02786624_g1	
	Target	Forward/Reverse primer 5′–3′	
Targeted mutation sequencing	Long Range GBA	CGACTTTACAAACCTCCCTG/CCAGATCCTATCTGTGCTGG	
	Nested GBA exon 6	CTAATGGCTGAACCGGATG/GGAAGTGGAACTAGGTTGAGG	
	Nested GBA exon 10–11	GTGGGTGACTTCTTAGATGAGG/CTTTAGTCACAGACAGCGTGTG	

germ layer test was conducted using Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems®), to differentiate into endoderm and ectoderm. To differentiate into mesoderm, a WNT pathway activator, CHIR99021 supplement (Stemcell™ Technologies) was used. The Base Media Supplement (50X) from the Stem Cell Functional Identification Kit (R&D Systems®) was used in all cases. The Stem Cell Functional Identification Kit (R&D Systems®) also contained antibodies, against Otx2, Brachyury and SOX17 (ectoderm, mesoderm and endoderm markers, respectively). In brief, seeded iPSCs were fixed in 4% paraformaldehyde (Sigma-Aldrich) for 20 min and washed with PBS. Subsequently, cells were permeabilized with 0.25% triton X-100 (Sigma-Aldrich) for 10 min, incubated with PBST with 1% BSA (Sigma-Aldrich) for 30 min and stained by standard immunofluorescence procedures. Cells were analysed on DM4000 M fluorescence microscope (Leica). Antibodies are listed in Table 2.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2019.101595.

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