University Library

A gateway to Melbourne's research publications

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:
Bozaoglu, K;Shern Lee, W;Haebich, KM;North, KN;Payne, JM;Lockhart, PJ

Title:
Generation of four iPSC lines from Neurofibromatosis Type 1 patients

Date:
2020-12-01

Citation:
Bozaoglu, K., Shern Lee, W., Haebich, K. M., North, K. N., Payne, J. M. & Lockhart, P. J. (2020). Generation of four iPSC lines from Neurofibromatosis Type 1 patients. Stem Cell Research, 49, https://doi.org/10.1016/j.scr.2020.102013.

Persistent Link:
https://hdl.handle.net/11343/294196

License:
CC BY-NC-ND

Stem Cell Research 49 (2020) 102013

Contents lists available at ScienceDirect

Stem Cell Research

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

Generation of four iPSC lines from Neurofibromatosis Type 1 patients

Kiymet Bozaoglu[a,b,*], Wei Shern Lee[a,b], Kristina M. Haebich[a,b], Kathryn N. North[a,b], Jonathan M. Payne[a,b,c], Paul J. Lockhart[a,b]

[a] Murdoch Children's Research Institute, Parkville, Australia
[b] Department of Paediatrics, University of Melbourne, Parkville, VIC, Australia
[c] Royal Children's Hospital, Parkville, VIC, Australia

A B S T R A C T

We describe the generation and characterisation of four human induced pluripotent stem cell (iPSC) lines from peripheral blood mononuclear cells (PBMC) from individuals with neurofibromatosis type (NF1). PBMC reprogramming was performed using a non-integrative Sendai virus containing the reprogramming factors OCT4, SOX2, MYC and KLF4. All iPSC lines exhibited a normal karyotype, and pluripotency was validated by flow cytometry (EPCAM, TRA-1-81, SSEA1 and CD9) and immunofluorescence (OCT4 and Nanog). Differentiation of the cells into the three embryonic germ layers was confirmed using immunofluorescence. These iPSC lines are a valuable pre-clinical resource to study the molecular mechanisms underlying NF1.

1. Resource Table:

Unique stem cell lines identifier	MCRIi020-A MCRIi021-A MCRIi022-A MCRIi023-A
Alternative names of stem cell lines	NF1_043.7 (MCRIi020-A) NF1_047.7 (MCRIi021-A) NF1_093.2 (MCRIi022-A) NF1_162.1 (MCRIi023-A)
Institution	Murdoch Children's Research Institute, Parkville, VIC, Australia
Contact information of distributor	A/Prof Paul Lockhart Paul.lockhart@mcri.edu.au
Type of cell lines	iPSC
Origin	Human
Cell Source	PBMC
Clonality	Clonal
Method of reprogramming	Transgene from Sendai Virus
Multiline rationale	same disease non-isogenic cell lines
Gene modification	No
Type of modification	N/A
Associated disease	Neurofibromatosis type 1
Gene/locus	17q11.2
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	04/2020
Cell line repository/bank	N/A
Ethical approval	This study was approved through the Human Research Ethics Committee of the Royal Children's Hospital (35118), Victoria, Australia.

2. Resource utility

Neurofibromatosis type 1 (NF1) is a neurogenetic condition associated with neurodevelopmental disorders. Understanding disease pathogenesis has been compromised by the inaccessibility of disease-relevant brain tissue. Pre-clinical models using patient-derived pluripotent stem cells (iPSCs) will enable disease modelling and drug screening platforms to identify new treatments and advance future clinical trials (Avior et al., 2016).

3. Resource details

NF1 is an autosomal dominant genetic condition caused by a diverse range of loss-of-function mutations in the *NF1* gene on chromosome 17q11.2. With a birth incidence of ~1 in 2,700, NF1 is one of the most common monogenetic conditions affecting children worldwide (Gutmann et al., 2017). Significant variation in disease severity is typically observed, with patients at an increased risk of developing a range of debilitating clinical manifestations involving the central and peripheral nervous systems, from benign and malignant tumour burden to cognitive dysfunction (Gutmann et al., 2017). Up to 80% of children with NF1 are affected by moderate-to-severe deficits in cognition and

* Corresponding author at: Murdoch Children's Research Institute, 50 Flemington Road Parkville, Victoria 3052, Australia.
E-mail address: kiymet.bozaoglu@mcri.edu.au (K. Bozaoglu).

https://doi.org/10.1016/j.scr.2020.102013
Received 16 September 2020; Accepted 26 September 2020
Available online 01 October 2020
1873-5061/ © 2020 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
NF1_043.7 (MCRIi020-A)	NF1_043.7	Female	7	Caucasian	N/A	Neurofibromatosis Type 1
NF1_047.7 (MCRIi021-A)	NF1_047.7	Male	9	Caucasian	N/A	Neurofibromatosis Type 1
NF1_093.2 (MCRIi022-A)	NF1_093.2	Male	13	Caucasian	N/A	Neurofibromatosis Type 1
NF1_162.1 (MCRIi023-A)	NF1_162.1	Female	9	Caucasian	N/A	Neurofibromatosis Type 1

Table 2
Characterisation and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1A
Phenotype	Qualitative analysis (Immunofluorescence)	Oct4, Nanog	Fig. 1 panel C
	Quantitative analysis (Flow cytometry)	TRA-1–81: > 91% SSEA4: > 84% CD9: > 98%	e.g. Fig. 1 panel B
Genotype	Karyotype (SNP Array)	Illumina Infinium GSA-24 v2.0	Submitted in archive with journal
Identity	Microsatellite PCR (mPCR) OR STR analysis [mandatory]	SNPduo comparative analysis performed to compare parental and derived clones	Submitted in archive with journal
		Identical genotypes (> 99.9%) for the entire genome, indicating cell lines are from the same individual	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Negative	Submitted in archive with journal
Differentiation potential	Embryoid body formation (immunofluorescence)	Ectoderm: MAP2 Endoderm: SOX17 Mesoderm: SMA	Fig. 1 panel D, E and F
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

behaviour, making it the most common complication experienced by this population during childhood (Hyman et al., 2005). There are currently no established biomarkers of cognitive deficits in children with NF1 and this is primarily due to gaps in our understanding of the neurobiological mechanisms underlying NF1. Whilst animal models have contributed to our understanding of NF1 and identified potential therapeutic treatments, translation into randomised clinical trials have not been successful (Payne et al., 2016). There is a need to discover early developmental biomarkers for NF1 since early treatment is more likely to improve functional connections in the developing brain (i.e., a particularly sensitive period for increased neuroplasticity), thereby leading to improved clinical outcomes. Patient derived iPSCs are a valuable tool to facilitate the molecular characterisation of NF1. They represent an opportunity to progress our understanding of the pathological processes in humans, advance future clinical trial design, and ultimately, improve clinical care of affected individuals.

This study describes the generation and characterisation of four human iPSC lines from peripheral blood mononuclear cells (PBMCs) from individuals with NF1. Blood samples were collected and PBMCs were isolated. iPSC lines were generated from the PBMCs using the Sendai virus carrying the Yamanaka reprogramming factors OCT4, SOX2, KLF4 and MYC (Table 1). All iPSC lines showed a normal karyotype (with 0.5 Mb resolution) and an identical genotype to their corresponding PBMC sample when analysed by array comparative genomic hybridisation. This analysis confirmed that no perturbations in genomic integrity had occurred during the reprogramming process (Supplementary 1). All iPSC lines were mycoplasma free. All characterisation and validation analyses are summarized in Table 2.

All lines displayed normal iPSC morphology, including prominent nucleoli, small and tightly packed cells, and a high nucleus to cytoplasm ratio (Fig. 1A). Pluripotency of the cells was determined using flow cytometry and immunofluorescence. Flow cytometry showed that > 90% of viable cells sorted positive for the pluripotency markers TRA-1-81, CD9, SSEA4 and EPCAM (Fig. 1B). In addition, immunofluorescence staining using the markers OCT4 and Nanog further verified pluripotency of the cells (Fig. 1C). All iPSC lines were able to form embryoid bodies *in vitro*, which expressed markers consistent with the development of the three germ layers ectoderm, mesoderm and endoderm. Specifically, immunofluorescence staining identified the ectoderm marker MAP2, endodermal marker SOX17 and the mesodermal marker SMA (Fig. 1 D, E and F respectively).

In summary, we have generated four iPSC lines that can be differentiated into neuronal and glial cells to study the neurobiological mechanisms of NF1. These models represent a valuable resource for future neurodevelopment and therapeutic discovery studies.

4. Materials and methods

4.1. PBMC isolation

Blood samples were collected from four individuals with NF1. These samples consisted of two males and two females. PBMC isolation was performed by diluting the blood 1:2 (vol:vol) in PBS/2% FBS and layering over Lymphoprep (StemCell Technologies) in SepMate™-15 tubes (StemCell Technologies). Samples were then centrifuged at 1,200 rcf for 10 min, transferred to a fresh tube, washed with PBS/2% FBS and centrifuged at 300 rcf for 10 min.

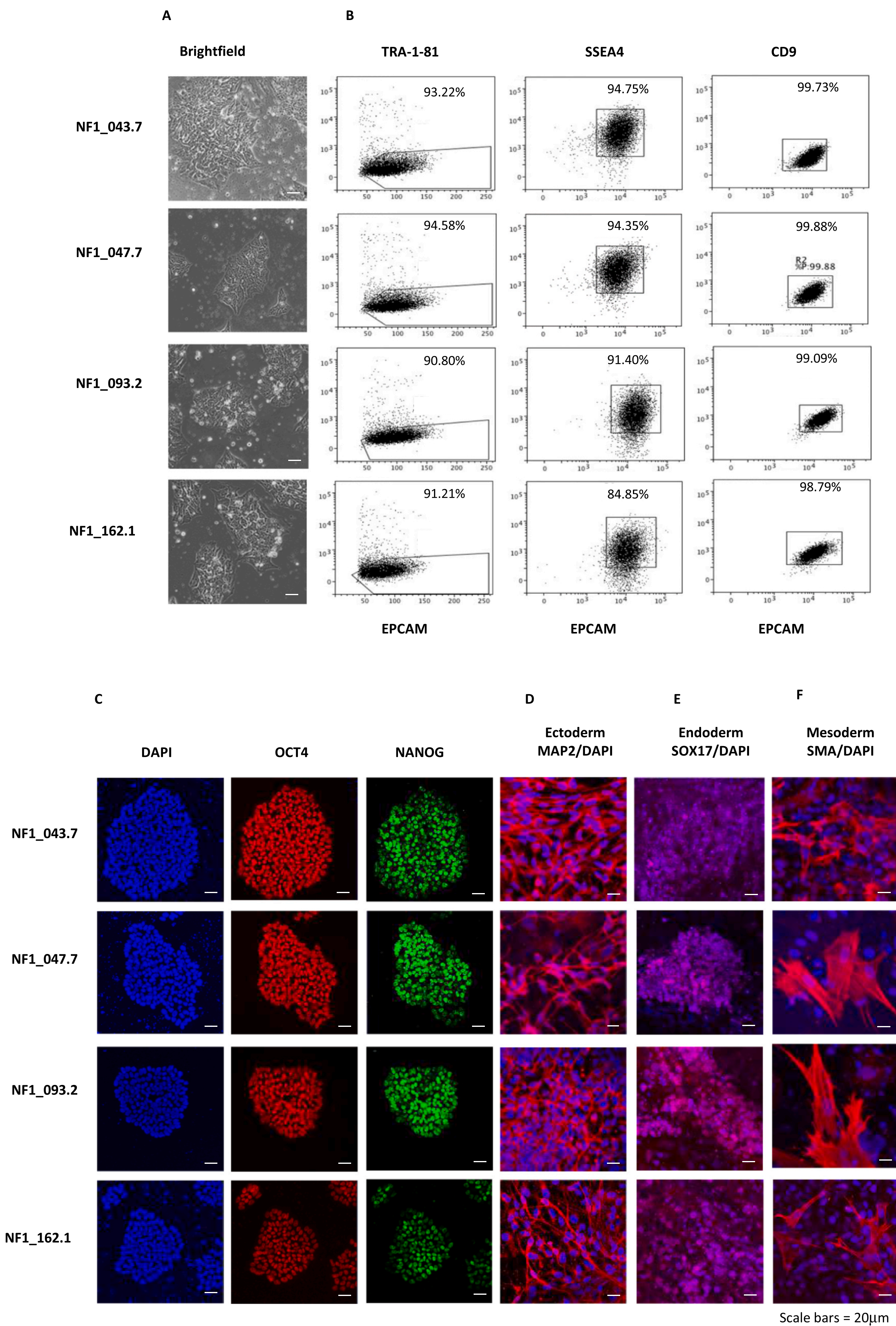

Fig. 1. Characterisation of the cell lines generated were performed using immunofluorescence and flow cytometry (Fig. 1).

4.2. iPSC generation

Reprogramming of PBMCs into iPSCs were performed using the Cytotune-iPS 2.0 Sendai Reprograming kit (ThermoFisher Scientific). Transduced cells were plated on culture dishes seeded with irradiated mouse embryonic fibroblasts (MEFs), and maintained in Knockout DMEM/20% Knockout serum replacer (ThermoFisher Scientific) supplemented with 50 ng/mL of FGF2 (Costa et al., 2008). iPSC colonies

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-Oct-4A monoclonal antibody (C30A3)	1:400	Cell Signaling Technology Cat#2840 RRID:AB_2167691
Pluripotency Markers	Nanog monoclonal antibody	1:400	BioLegend Cat# 674202, RRID:AB_2564574
Pluripotency Markers	Mouse anti-SSEA4 monoclonal antibody	1:400	Millipore Cat#MAB4304 RRID:AB_177629
Pluripotency Markers	Alexa Fluor 647 anti-human TRA-1-81 antibody	1:100	BioLegend Cat# 330706, RRID:AB_1089242
Pluripotency Markers	PE conjugated anti-human CD326 (EPCAM) antibody, Clone EBA-1	1:30	BD Biosciences Cat# 347198, RRID:AB_400262
Pluripotency Markers	PE/Cy7 anti-human SSEA-4 antibody	1:100	BioLegend Cat# 330420, RRID:AB_2629631
Pluripotency Markers	Mouse Anti-CD9 Monoclonal Antibody, FITC Conjugated, Clone M − L13	1:10	BD Biosciences Cat# 555371, RRID:AB_395773
Differentiation Markers	Mouse Anti-MAP-2 (MAP-2A and − 2B) Monoclonal antibody, Unconjugated, Clone AP20	1:500	Millipore Cat# MAB3418, RRID:AB_94856
Differentiation Markers	Goat anti SOX17 polyclonal Antibody	1:50	Santa Cruz Biotechnology Cat# sc-17355, RRID:AB_2239898
Differentiation Markers	Monoclonal Mouse Anti Human Smooth Muscle Actin antibody	1:25	Agilent Cat# M0851, RRID:AB_2223500
Secondary Antibodies	Goat Anti-Mouse IgG (H + L) Highly Cross-adsorbed Antibody, Alexa Fluor 594 Conjugated	1:1000	Molecular Probes Cat# A-11032, RRID:AB_2534091
Secondary Antibodies	Goat Anti-Chicken IgG (H + L) Antibody, Alexa Fluor 488 Conjugated	1:1000	Molecular Probes Cat# A-11039, RRID:AB_142924
Secondary Antibodies	Donkey Anti-Goat IgG (H + L) Antibody, Alexa Fluor 647 Conjugated	1:1000	Molecular Probes Cat# A-21447, RRID:AB_141844
Primers			
	Target	**Forward/Reverse primer (5′-3′)**	
	N/A	N/A	

were mechanically isolated and expanded as previously described (Costa et al., 2008). The lines were adapted to feeder free bulk culture on plates coated with Vitronectin (Stemcell technologies) in Essential 8 medium (ThermoFisher Scientific). All cells were cultured at 37 °C with 10% CO_2.

4.3. Karyotyping

Karyotyping was performed using the Illumina Infinium GSA-24 v2.0 at passage number P3 + 7 (NF1_043.7, NF1_047.7, NF1_162.1) or P3 + 8 (NF1_093.2) and compared to the human reference sequence hg19/GRCh37 (Feb 2009). All of the lines demonstrated a normal karyotype. SNP array comparisons of parental PBMC and iPSC lines were performed using SNPDuo (http://pevsnerlab.kennedykrieger.org/SNPduo/). No differences were detected between the original PBMC sample and its corresponding iPSC line.

4.4. Mycoplasma testing

The absence of mycoplasma contamination in the iPSC lines were confirmed by PCR (Cerberus Sciences, Adelaide, Australia).

4.5. Flow cytometry

iPSCs were harvested, filtered and incubated with conjugated antibodies (Table 3) for 15 min on ice. Cells were then stained with Propidium Iodide (Sigma) and analysed with a LSR Fortessa Cell Analyser (BD Bioscience).

4.6. Embryoid body (EB) formation

iPSCs were seeded in ultra-low adherence 96 well plates and cultured in E8 media (Stemcell technologies) with 0.5% polyvinyl alcohol (Sigma) for 24 h. Subsequently, the cells were cultured for 2 weeks in E8 media, refreshed every 2–3 days, then plated onto vitronectin-coated glass coverslips and cultured in E8 medium for 3 weeks.

4.7. Immunofluorescence (IF)

Cells were fixed with 4% Paraformaldehyde for 10 min, permeabilised in 0.2% Triton X-100 (Sigma) for 10 min and blocked in 2% Bovine Serum Albumin (Life Technologies) for 60 min at room temperature. Cells were then incubated with primary antibodies at 4 °C overnight, followed by an incubation with secondary antibodies for 60 min at room temperature (Table 3). The coverslips were mounted on slides with DAPI to stain the nuclei (VectorLabs). Images were captured with an LSM 780 confocal microscope running Zen Black software, an Axio Observer.Z1 microscope with an Axiocam 506 mono camera running Zen Blue software (Carl-Zeiss).

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.

Acknowledgements

We would like to thank the participants and their families for participating in our research. This research was partly funded by the Barney neurofibromatosis fund. K.B is supported by an E.H. Flack Fellowship, P.J.L is supported by the Vincent Chiodo Foundation, and J.M.P is supported by a MCRI Clinician Scientist Fellowship. Additional infrastructure funding to the MCRI was provided by the Australian Government NHMRC Independent Research Institute Infrastructure Support Scheme and the Victorian Government's Operational Infrastructure Support Program. The MCRI iPSC Core Facility is supported by the Stafford Fox Medical Research Foundation

References

Avior, Y., Sagi, I., Benvenisty, N., 2016. Pluripotent stem cells in disease modelling and drug discovery. Nat. Rev. Mol. Cell Biol. 17, 170–182.
Gutmann, D.H., Ferner, R.E., Listernick, R.H., Korf, B.R., Wolters, P.L., Johnson, K.J., 2017. Neurofibromatosis type 1. Nat. Rev. Dis. Primers 3, 17004.
Hyman, S.L., Shores, A., North, K.N., 2005. The nature and frequency of cognitive deficits in children with neurofibromatosis type 1. Neurology. 65, 1037–1044.
Payne, J.M., Barton, B., Ullrich, N.J., Cantor, A., Hearps, S.J., Cutter, G., et al., 2016. Randomized placebo-controlled study of lovastatin in children with neurofibromatosis type 1. Neurology. 87, 2575–2584.
Costa M, Sourris K, Hatzistavrou T, Elefanty AG, Stanley EG. Expansion of human embryonic stem cells in vitro. Curr Protoc Stem Cell Biol. 2008;Chapter 1:Unit 1C -C 7.