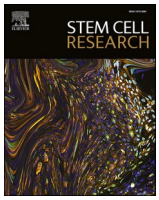
Stem Cell Research 80 (2024) 103524

Contents lists available at ScienceDirect

Stem Cell Research

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



Lab Resource: Single Cell Line

Generation of two isogenic patient-derived human-induced pluripotent stem cell clones with 6q27 deletion

Luca Bianchini [a,b], Laura Sieber [a,b], Ruba Hammad [c,d,e,f], Richard Schäfer [c,d,e], Lena M. Kutscher [a,b,*]

[a] Developmental Origins of Pediatric Cancer Junior Research Group, German Cancer Research Center (DKFZ), Heidelberg, Germany
[b] Hopp Children's Cancer Center (KiTZ), Heidelberg, Germany
[c] Institute for Transfusion Medicine and Gene Therapy, Medical Center-University of Freiburg, Freiburg, Germany
[d] Center for Chronic Immunodeficiency (CCI), Medical Center-University of Freiburg, Freiburg, Germany
[e] Freiburg iPS Core, Medical Center-University of Freiburg, Freiburg, Germany
[f] Faculty of Biology, University of Freiburg, Freiburg, Germany

A B S T R A C T

We generated two human induced pluripotent cell (hiPSC) isogenic clones from an 11-year-old patient with 6q27 deletion syndrome. The heterozygous deletion encompasses approximately 240 kilobases, affecting 6 genes (promoter region of *WDR27,* coding regions of *C6orf120, PHF10, DYNLT2, ERMARD, LINC00242*). The patient suffered from epilepsy, psychosocial retardation, and a metabolic disorder. The patient also had a history of SHH-medulloblastoma as an infant. The generated hiPSCs represent a useful tool for modelling 6q27 deletion syndrome *in vitro* and understanding the molecular basis of the disorder.

Resource Table

Unique stem cell lines identifier	DKFZi001-A DKFZi001-B
Alternative name(s) of stem cell lines	N/A
Institution	German Cancer Research Center (DKFZ)
Contact information of distributor	Lena Kutscher (l.kutscher@kitz-heidelberg.de)
Type of cell lines	iPSC
Origin	human
Additional origin info required	Age: 11 Sex: Male
Cell Source	Total peripheral blood mononuclear cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Sendai virus
Genetic Modification	NO
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss	qPCR
Associated disease	6q27 Syndrome
Gene/locus	Heterozygous deletion of chr6q27:170,109,037-170,348,671 (GRCh37/hg19)
Date archived/stock date	N/A
Cell line repository/bank	https://hpscreg.eu/cell-line/DKFZi001-A https://hpscreg.eu/cell-line/DKFZi001-B
Ethical approval	The ethics committee of the Medical Faculty of Heidelberg (S-695/2020) approved the study and informed consent was obtained from the patient and patient's parents.

(*continued on next column*)

Resource Table (*continued*)

1. Resource utility

Subtelomeric chromosome 6q27 deletions have been previously linked to neurodevelopmental disorders (Engwerda et al., 2023), but so far, few studies provide molecular insights into these processes. The generation of these patient-derived hiPSC clones provide a powerful tool to model such developmental disorders and gain molecular insights for better understanding disease pathology.

2. Resource details

A patient was identified who was diagnosed with infant SHH-medulloblastoma, a pediatric brain tumor arising from the cerebellum (Table 1). A coincident 6q27 heterozygous germline deletion (~240 kb) was also identified. Other comorbidities of the patient included epilepsy, psychosocial retardation, and metabolic disorder, consistent with 6q27

* Corresponding author at: Developmental Origins of Pediatric Cancer Junior Research Group, German Cancer Research Center (DKFZ), Heidelberg, Germany.
E-mail address: l.kutscher@kitz-heidelberg.de (L.M. Kutscher).

https://doi.org/10.1016/j.scr.2024.103524
Received 25 June 2024; Accepted 30 July 2024
Available online 31 July 2024

deletion syndrome (Zhou, 2014). This deletion affected the promoter of *WDR27*, and the entire coding sequence of *C6orf120*, *PHF10*, *DYNLT2*, *ERMARD*, *LINC00242* (Fig. 1A). Subtelomeric 6q deletions have been reported to be highly heterogeneous, both in size of the genomic region that is lost, and the clinical manifestations of the accompanying syndrome (Hanna, 2019; Lee et al., 2011; Ognibene et al., 2020).

The patient-derived iPSC isogenic clones were reprogrammed from PBMCs using Sendai virus, with *OCT3/4*, *SOX2*, *c-MYC*, and *KLF4* used as reprogramming factors. Extensive quality control was performed on the two most promising clones and is reported here (DKFZi001-A, DKFZi001-B). Both hiPSC clones had the characteristic morphology, with no spontaneous differentiation in mTeSR+ media (Fig. 1B). Both lines expressed the pluripotency markers OCT3/4, NANOG, SOX2, and SSEA4 (Fig. 1C). Pluripotency was quantified by flow cytometry, showing a high proportion of cells expressing pluripotency markers (OCT3/4, SOX2, SSEA4, TRA-1-60), and a low percentage of cells expressing the differentiation marker SSEA1 (Fig. 1D). Of note, DKFZi001-A showed a higher proportion of SSEA1-positive cells, although morphologically no differentiation was observed. We confirmed that no unwanted chromosomal alterations were induced during reprogramming using molecular karyotyping (Fig. 1E). Cell identity of the clones was identical to the starting material, as confirmed by STR analysis of 16 genomic loci. Both hiPSC clones were capable of differentiation to all three germ layer lineages, as shown by the expression of the ectodermal markers PAX6 and OTX2, mesodermal makers TBXT and CD56, and endodermal makers SOX17 and FOXA2 (Fig. 1F). The two iPSC clones were maintained mycoplasma free (Supplementary Fig. 1A) and the original programming factors were absent as assessed by qPCR (Supplementary Fig. 1B).

In summary, we generated two human-induced pluripotent cell clones from a patient carrying a heterozygous 6q27 deletion. Pluripotency was confirmed by the expression of pluripotency markers and differentiation potential to the three germ layers. These new cell clones provide a promising reagent to increase the understanding of how 6q27 deletions affect cell biology and brain development.

3. Materials and methods

3.1. Reprogramming of human PBMCs

Patient-derived PBMCs were reprogrammed using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Invitrogen, #A16517), following manufacturer's instructions for feeder-free reprogramming. Briefly, PBMCs were activated in RPMI medium supplemented with IL2 (100U/mL), IL15 (50U/mL), IL7 (25U/mL), and Immunocult (Stemcell, #10970). 500,000 cells were transduced (MOI6) with the Sendai virus mix; emerging colonies were manually picked and cultured in mTeSR+ media (Stem Cell Technologies, #100-0276).

3.2. Maintenance of the human iPSC clones

hiPSC were maintained in mTeSR+ in Matrigel-coated plates (Corning, #354277) at 37 °C 5 % CO_2. Once cells were ~ 80 % confluence, they were split using ReLeSR (Stem Cell Technologies, #100-0484) for 2 min at 37 °C. Colonies were detached by shaking, and passaged at 1:20 ratio. hiPSCs were cryopreserved as colonies in Bambanker (Nippon Genetics, #BB03). All experiments have been performed on passage 6 hiPSCs.

3.3. Tri-lineage differentiation

hiPSCs were differentiated to the three germ layers using the STEMdiff Trilineage Differentiation Kit (Stem Cell Technologies, #05230), following the manufacturer's instructions. Antibody details are available in Table 2.

3.4. Immunofluorescence staining

Cells were fixed in 4 % PFA for 15 min, washed with DPBS, and blocked for 1 h at room temperature with staining solution (10 % Donkey serum, 0.1 % Triton X-100 in DPBS). Primary antibodies were diluted in staining solution and incubated overnight at 4 °C. After washing with staining solution for 30 min, secondary antibodies were incubated in staining solution with DAPI (1 mg/ml) for one hour at room temperature. After washing with staining solution for 30 min, coverslips were mounted on slides. Images were acquired at Zeiss LSM900 Airyscan2, and processed using FIJI.

3.5. Flow cytometry

Cells were fixed in Fix and Permeabilization Solutions (Thermo Fisher, #GAS003), following manufacturer's instructions. $5x10^5$ cells were stained with the antibody diluted in FACS buffer (2 % FBS in DPBS) for 1 h at 4 °C. After two washes with FACS buffer, fluorescent signal was acquired using a FACSCanto II (Becton Dickinson). Antibody details are available in Table 2.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Bright field microscopy	Normal morphology	Fig. 1, panel B
Phenotype	Qualitative analysis (Immunofluorescence)	Expression of pluripotency markers (OCT3/4, NANOG, SOX2, SSEA4)	Fig. 1, panel C
	Quantitative analysis (Flow cytometry)	High percentage of cells expressing pluripotency markers (OCT3/4, SOX2, SSEA4, TRA-1-60), and low percentage of cells expressing differentiated marker (SSEA1)	Fig. 1, panel D
Genotype	Molecular Karyotype	46,XY,-6q27 Effective resolution 31 kb	Fig. 1, panel E
Identity	STR analysis	16 STR sites analyzed, all matching between PBMCs and the two hiPSC clones	Submitted in archive with journal
Mutation analysis	Sequencing	Heterozygous deletion (chr6:170,109,037-170,348,671 GRCh37/hg19)	Fig. 1, panel A
	WGS	N/A	
Microbiology and virology	Mycoplasma	Negative	Supplementary Fig. 1, panel A
Differentiation potential	Tri-lineage directed differentiation (Immunofluorescence)	Ectoderm: PAX6, OTX2 Endoderm: SOX17, FOXA2 Mesoderm: TBXT, CD56	Fig. 1, panel 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

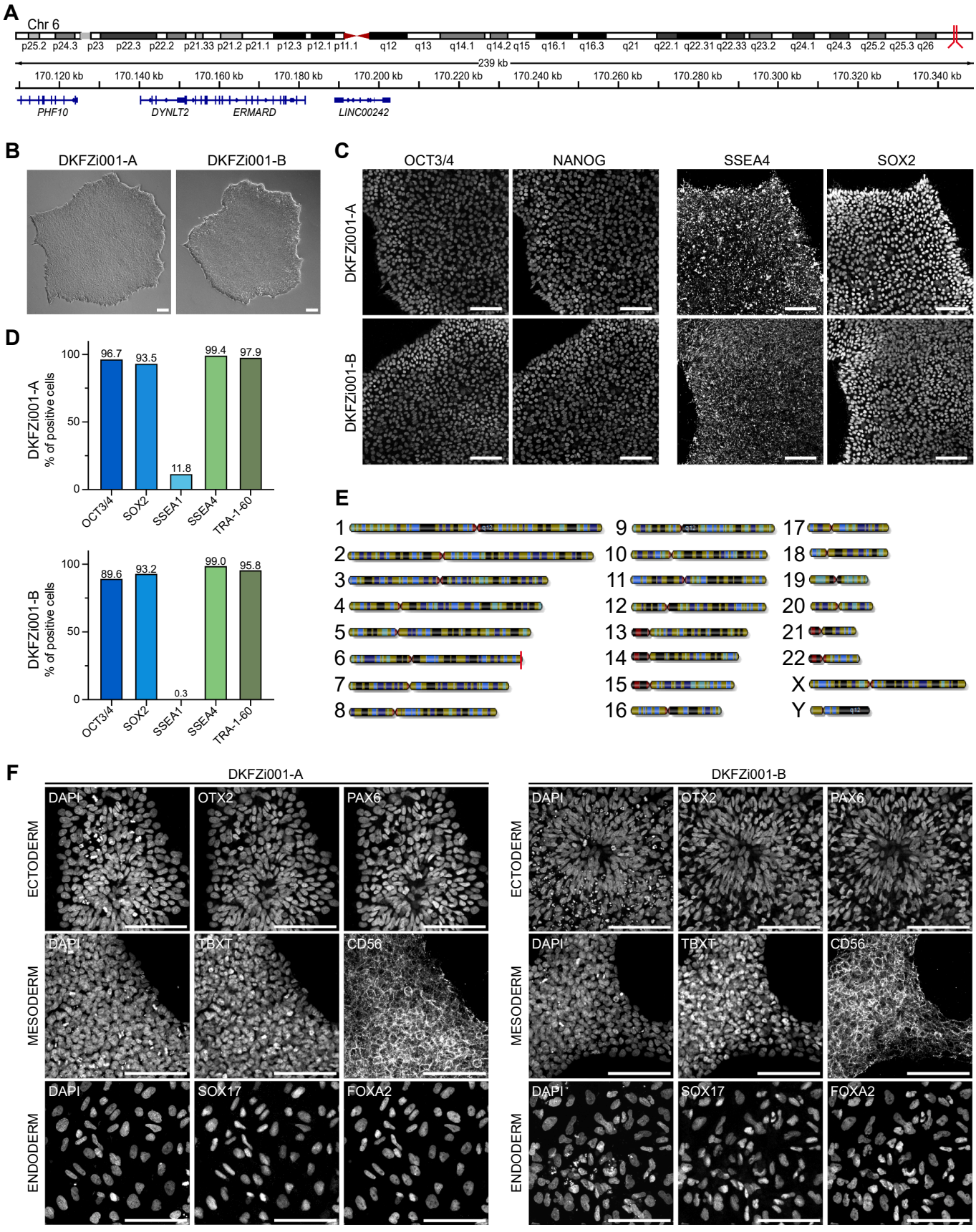


Fig. 1. Characterization of two isogenic patient-derived iPSC clones with 6q27 deletion (100 μm scalebars).

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency marker	Rabbit anti-NANOG	1:500	Cell Signaling Technology Cat# 4903	AB_10559205
Pluripotency marker	Mouse anti-OCT4	1:500	Santa Cruz Biotechnology Cat# sc-5279	AB_628051
Pluripotency marker	Rabbit anti-SOX2	1:500	Cell Signaling Technology Cat# 23064	AB_2714146
Pluripotency marker	Mouse anti-SSEA4	1:500	Cell Signaling Technology Cat# 4755	AB_1264259
Differentiation Marker	Rabbit anti-PAX6	1:500	BioLegend Cat# 901301	AB_2565003
Differentiation Marker	Goat anti-OTX2	1:500	R&D Systems Cat# AF1979	AB_2157172
Differentiation Marker	Goat anti-TBXT	1:500	R&D Systems Cat# AF2085	AB_2200235
Differentiation Marker	Mouse anti-CD56	1:500	BioLegend Cat# 318304	AB_604100
Differentiation Marker	Rabbit anti-FOXA2	1:500	Abcam Cat# ab108422	AB_11157157
Differentiation Marker	Mouse anti-SOX17	1:500	BioLegend Cat# 698502	AB_2687318
Secondary antibody	AF488 Goat Anti-Rabbit IgG	1:500	Molecular Probes Cat# A-11008	AB_143165
Secondary antibody	AF568 Goat Anti-Mouse IgG	1:500	Molecular Probes Cat# A-11004	AB_2534072
Secondary antibody	AF568 Donkey Anti-Goat IgG	1:500	Molecular Probes Cat# A-11057	AB_2534104
Primers				
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Reprogramming factor (qPCR)	SeV-cMyc	237 bp	TAACTGACTAGCAGGCTTGTCG/TCCACATACAGTCCTGGATGATGATG	
Reprogramming factor (qPCR)	SeV-KOS	528 bp	ATGCACCGCTACGACGTGAGCGC/ACCTTGACAATCCTGATGTGG	
Reprogramming factor (qPCR)	SeV-Klf4	410 bp	TTCCTGCATGCCAGAGGAGCCC/AATGTATCGAAGGTGCTCAA	
Reprogramming factor (qPCR)	SeV	181 bp	GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC	
House-keeping gene (qPCR)	TBP	108 bp	CCCATGACTCCCATGACC/TTTACAACCAAGATTCACTGTGG	

3.6. Molecular Karyotyping and STR profiling

Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, #69504). Molecular karyotyping was performed using Infinium™ HumanCytoSNP-12 v2.1 Beadchip kits (Illumina), following manufacturer's instructions. Data was analysed with GenomeStudio 2. STR profiling was performed using a AmpFLSTR™ Identifiler™ Kit (Applied Biosystems, #4427368) by Multiplexion GmbH.

3.7. Reverse transcription and qPCR

Total RNA was extracted using the RNeasy Kit (Qiagen, #74004), and 600 ng of RNA was reverse transcribed with random hexamers using the SuperScript RT Kit (Thermo Fisher, #18091050), following manufacturer's instructions. Quantitative PCR was performed using SYBR Green PCR Master Mix (Thermo, #4309155). Quantification of the transcripts was performed using the ΔΔCt method. Primer sequences are available in Table 2.

3.8. Mycoplasma

Absence of mycoplasma was confirmed at banking via PCR with the PCR Mycoplasma Detection Kit (Abm, #ABM-G238), following manufacturer's instructions. Mycoplasma detection was then performed routinely every month, and cultures were maintained as mycoplasma free.

CRediT authorship contribution statement

Luca Bianchini: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Laura Sieber:** Methodology. **Ruba Hammad:** Methodology. **Richard Schäfer:** Project administration, Supervision. **Lena M. Kutscher:** Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

No competing interests declared.

Acknowledgements

We thank Dr. Anika Schmith for coordinating the research stay at the Freiburg iPS core. This work was supported by an Young IBRO Maternity/Parenthood Grant and DFG Individual Grant (#497317859) to L.M. K. We thank the following core facilities of the DKFZ: light microscopy, microarray, next generation sequencing, and flow cytometry.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2024.103524.

References

Engwerda, A., Kerstjens-Frederikse, W.S., Corsten-Janssen, N., Dijkhuizen, T., van Ravenswaaij-Arts, C.M.A., 2023. The phenotypic spectrum of terminal 6q deletions based on a large cohort derived from social media and literature: a prominent role for DLL1. Orphanet. J. Rare Dis. 18, 59. https://doi.org/10.1186/s13023-023-02658-w.

Hanna, M.D., et al., 2019. Defining the critical region for intellectual disability and brain malformations in 6q27 microdeletions. Mol. Syndromol. 10, 202–208. https://doi.org/10.1159/000501008.

Lee, J.Y., Cho, Y.H., Hallford, G., 2011. Delineation of subtelomeric deletion of the long arm of chromosome 6. Ann. Hum. Genet. 75, 755–764. https://doi.org/10.1111/j.1469-1809.2011.00675.x.

Ognibene, M., Morini, M., Garaventa, A., Podesta, M., Pezzolo, A., 2020. Identification of a minimal region of loss on chromosome 6q27 associated with poor survival of high-risk neuroblastoma patients. Cancer Biol. Ther. 21, 391–399. https://doi.org/10.1080/15384047.2019.1704122.

Zhou, L., et al., 2014. Delineation variable genotype/phenotype correlations of 6q27 terminal deletion derived from dic(6;18)(q27;p10). Mol. Cytogenet. 7, 78. https://doi.org/10.1186/s13039-014-0078-3.