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Lab resource: Stem Cell Line

Generation of the Niemann–Pick type C2 patient-derived iPSC line AKOSi001-A

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A B S T R A C T

Niemann-Pick disease Type C (NPC) is a rare progressive neurodegenerative disorder with an incidence of 1:120,000 caused by mutations in the *NPC1* or *NPC2* gene. Only 5% of NPC patients suffer from mutations of the *NPC2* gene. Here we demonstrate the generation of a Niemann–Pick disease Type C2 (NPC2) patient-derived induced pluripotent stem cell line. This cell line is capable to differentiate into derivatives of the neuronal lineage, providing a valuable tool to study pathogenic mechanisms of NPC2.

Resource table

Unique stem cell line identifier	AKOSi001-A
Alternative name(s) of stem cell line	iPS GM18455-1
Institution	Translational Neurodegeneration Section "Albrecht-Kossel" Department of Neurology University Medical Center Rostock, Rostock, Germany
Contact information of distributor	Dr. Moritz Frech
Type of cell line	HYPERLINK "mailto: moritz.frech@med.uni-rostock.de iPSC
Origin	Human
Additional origin info	Age: unknown Sex: male Ethnicity: unknown
Cell source	Fibroblasts
Clonality	Clonal
Method of reprogramming	retroviral, Sox2, KLF4, Oct4, c-myc
Genetic modification	Yes
Type of modification	Hereditary
Associated disease	Niemann–Pick disease Type C2
Gene/locus	*NPC2* gene locus: allele 1 carries a substitution (G > T) at nucleotide 58 (c.58G > T) in exon 1, resulting in a nonsense mutation at codon 20 [E20X (GLU20TER)]; allele 2 carries a substitution (G > T) at nucleotide 140 (c.140G > T) in exon 2, resulting in a missense mutation at codon 47 [C47F (CYS47PHE)].
Method of modification	N/A
Name of transgene or resistance	Oct4, Sox2, KLF4,c-myc
Inducible/constitutive system	N/A
Date archived/stock date	2015
Cell line repository/bank	N/A
Ethical approval	Fibroblasts were obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research

1. Resource utility

No iPSC model system based on NPC2 patients´ fibroblasts was described before. The generated iPS cell line AKOSi001-A is applicable for disease modelling of NPC2, studying pathogenic mechanism and

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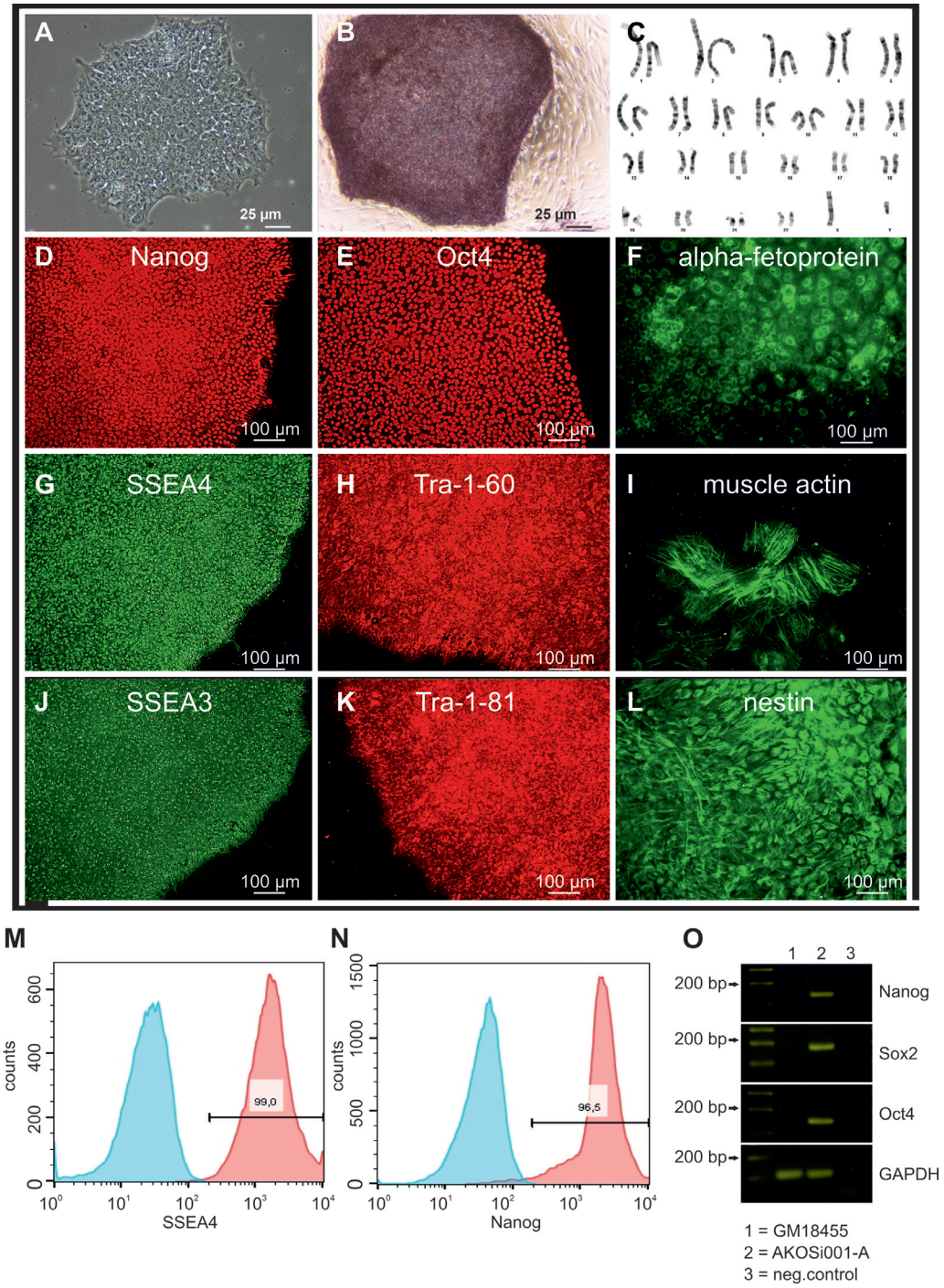

Fig. 1. Characterization of AKOSi001-A iPSC line.

drug development.

2. Resource details

Niemann-Pick disease type C (NPC) is an autosomal recessive lysosomal lipid storage disorder due to mutations in the *NPC1* or the *NPC2* gene. The incidence of NPC is approximately 1:120,000, wherein 95% of the cases show mutations in the *NPC1* gene and 5% in the *NPC2* gene (Vanier, 2010). NPC1 and NPC2 protein act in a cooperative manner, excluding cholesterol from the lumen of late endosomes and lysosomes. Thus, malfunction of one of these proteins results not only in a comparable cellular phenotype, but also in comparative clinical manifestations. Disease modelling of NPC is currently limited to NPC1 model systems and no models based on patient-derived iPSCs for NPC2 mutations are available. Here we describe the generation of a NPC2 patient-specific induced pluripotent stem cell line. Reprogramming was done by retroviral transduction using Sox2, KLF4, Oct4 and c-myc. Fibroblasts (GM18455, Coriell Institute for Medical Research, USA) of a NPC2 patient carrying the compound heterozygous mutation c.58G > T; c.140G > T were reprogrammed. IPSC colonies appeared approx. 14 days after reprogramming. Colonies demonstrated typical embryonic stem cell-like morphology, e.g. showing a sharp border with round to oval shape and a high nuclear to cytoplasm ratio (Fig. 1A). Mechanically isolated colonies were expanded to iPSC lines on irradiated mouse embryonic fibroblasts and later also on matrigel. IPSC colonies demonstrated alkaline phosphatase expression (Fig. 1B). The

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of cell line normal	Fig. 1 panel A
Phenotype	Qualitative analysis Immunocytochemistry	Staining of pluripotency markers alkaline phosphatase, marker: Nanog, Oct4, SSEA4, Tra-1-60, SSEA3, Tra-1-81	Fig. 1 panel D, E, G, H, J, K
	Quantitative analysis Immunocytochemistry by fluorescence intensity analysis	% of positive cells: Nanog: 95.5% ± 0.03% Oct4: 99.1% ± 0.01% SSEA4: 99.55% ± 0.05% SSEA3: 92.8% ± 0.03%	Fig. 1 panel D, E, G, J (representative)
	Quantitative analysis by Flow Cytometry	% of positive cells: Nanog: 96.5% Oct4: 90.6% SSEA3: 61.7% SSEA4: 99.0% Tra-1-60: 87.2% Tra-1-81: 85.8%	Fig. 1 panel M, N (representative)
	Qualitative analysis: RT-PCR	Expression of Pluripotency genes: Nanog, Sox2, Oct4	Fig. 1 panel O
Genotype	Karyotype (G-banding) and resolution	46XY, Resolution 450-500	Fig. 1 panel C
Identity	Microsatellite PCR (mPCR) OR	not performed	N/A
	STR analysis	16 loci tested, 16 loci matched	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	confirmed	Suppl. Fig. 1B,C
	Southern Blot OR WGS	not performed	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR: negative	Suppl. Fig. 1A
Differentiation potential	Embryoid body formation	Expression of genes in embryoid bodies:alpha-fetoprotein, muscle actin and nestin	Fig. 1 panel F, I, L
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	not performed	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	not performed	N/A
	HLA tissue typing	not performed	N/A

karyotype of the cells displayed no obvious aberrations (Fig. 1C). STR analysis confirmed that donor cells and derived iPS cells are identical. Targeted NGS sequencing revealed a similar single nucleotide variants (SNV) pattern of the compared samples showing two SNVs in NPC2 with highly comparable allele frequencies (Suppl. Table 2). The endogenous expression of several transcription factors and surface markers was determined by immunocytochemistry, FACS analysis and RT-PCR. IPSCs showed expression of the transcription factors Nanog and Oct4 (Fig. 1D,E). The glycosphingolipids SSEA3 (Fig. 1J) and SSEA4 (Fig. 1G) were expressed as well as the keratan sulfate antigens Tra-1-60 (Fig. 1H) and Tra-1-81 (Fig. 1K). FACS analysis was used to determine amounts of cells positive for e.g. SSEA4 (Fig. 1M) or Nanog (Fig. 1N, see also Table 1). Gene expression of Nanog, Oct4 and Sox2 was demonstrated in iPSCs but not in fibroblasts (Fig. 1. O). Pluripotency was further proven by spontaneous differentiation into embryoid bodies (EB). EBs contained cells of all three germ layers. Alpha-fetoprotein (Fig. 1F), muscle actin (Fig. 1I) and nestin (Fig. 1L) were used to demonstrate the capability of the cells to differentiate into cells of the endoderm, ectoderm, and mesoderm, respectively.

3. Materials and methods

3.1. Reprogramming of fibroblasts

Retroviral particle were used to transfect fibroblasts with Sox2, Oct4, KLF4, and c-myc, to reprogram patient-derived fibroblasts accordingly to Peter et al. (2017).

3.2. Cell culture

Cells were cultured accordingly to Peter et al. (2017) at 37 °C/5% CO_2. IPSCs, cultured on feeder cells, were kept in iPSC-medium (DMEM/F-12, 20% knockout serum replacement, 1% penicillin/streptomycin, 1% GlutaMAX, 1% MEM non-essential amino acids, 0.2% 2-mercaptoethanol, 15 ng/ml FGF-2 (Amsbio)). IPSCs cultured on Matrigel (Corning) were kept in mTeSR1 (Stemcell Technologies).

3.3. Karyotyping

G-Banding karyotyping was done by Cell Guidance Systems (Cell Guidance Systems, Moneta Building, Babraham Research Campus, Cambridge CB22 3AT, UK, www.cellgs.com). Colonies (passage number 15) were incubated with colcemid (10 µg/ml) overnight. IPSCs were harvested by trypsin treatment (0.25%) and treated with 0.075 M KCl. Cells were fixed with methanol/acetic acid (3:1) and 20 metaphases were analyzed, showing no apparent structural abnormalities.

3.4. Alkaline phosphatase staining

IPSCs were fixed (ice-cold methanol for 10 min) and incubated for 15 min with staining solution (75% distilled water, 10% sodium chloride solution (1 M), 10% Tris solution (1 M, pH 9.8), 5% magnesium chloride solution (1 M), NBT/BCIP solution (1:50, Roche)).

3.5. Immunocytochemistry

Cells were fixed for 15 min (4% paraformaldehyde) and subsequently incubated with 0.3% Triton X-100 and 5% normal goat serum (Dako) for 30 min at room temperature. Primary antibodies were added for 2 h in 1% normal goat serum. Secondary antibodies were incubated 1 h in phosphate buffered solution.

3.6. Flow cytometry

IPSCs were dissociated using Gentle Cell Dissociation Reagent (STEM CELL Technologies). For intracellular pluripotency markers Oct4 and Nanog, the True-Nuclear™ Transcription Factor Buffer Set (Biolegend) was used for fixation and permeabilization. Cells were incubated with fluorophore-conjugated antibodies for 1 h at 4 °C. Cells were analyzed using a FACSCalibur system (BD Biosciences). FlowJo software version 10.5.3 was used for graphical analysis.

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency marker	Mouse anti-Nanog	1:100	ReproCELL Incorporated Cat# RCAB004P-F, RRID:AB_1,560,380
Pluripotency marker	Rabbit anti-Oct4	1:100	Stemgent Cat# 09-0023, RRID: AB_2167689
Pluripotency marker	Mouse anti-SSEA4	1:100	Stemgent Cat# 09-0006, RRID:AB_1,512,169
Pluripotency marker	Mouse anti-SSEA3	1:100	Stemgent Cat# 09-0014, RRID:AB_151,216
Pluripotency marker	Mouse anti-Tra-1-60	1:100	Stemgent Cat# 09-0010, RRID:AB_1,512,170
Pluripotency marker	Mouse anti-Tra-1-81	1:100	Stemgent Cat# 09-0011, RRID:AB_1,512,171
Pluripotency marker	Alexa Fluor® 647 anti-Nanog Mouse IgG1, κ	1:50	BioLegend Cat# 674,210, RRID:AB_2,650,619
Pluripotency marker	Alexa Fluor® 488 anti-Oct4, Mouse IgG2b, κ	1:20	BioLegend Cat# 653,705, RRID:AB_2,562,250
Pluripotency marker	Alexa Fluor® 488 anti-human SSEA-4, Mouse IgG3, κ	1:500	BioLegend Cat# 330,411, RRID:AB_1,089,199
Pluripotency marker	PerCP/Cy5.5 anti-human/mouse SSEA-3, Rat IgM, κ	1:20	BioLegend Cat# 330,323, RRID:AB_2,728,281
Pluripotency marker	PE anti-human TRA-1-60-R, Mouse IgM, κ	1:20	BioLegend Cat# 330,609, RRID:AB_1,279,447
Pluripotency marker	Alexa Fluor® 647 anti-human TRA-1-81, Mouse IgM, κ	1:20	BioLegend Cat# 330,705, RRID:AB_1,089,245
Differentiation marker	Mouse anti-nestin	1:100	R and D Systems Cat# MAB1259, RRID:AB_2,251,304
Differentiation marker	Mouse anti-alpha fetoprotein	1:500	Sigma-Aldrich Cat# A8452, RRID:AB_258,392
Differentiation marker	Mouse anti-smooth muscle actin	1:50	Agilent Dako Cat# M0851, RRID:AB_2,223,500
Secondary antibody	Alexa Fluor 488, goat anti-mouse	1:500	Thermo Fisher Scientific Cat# R37120, RRID:AB_2,556,548
Secondary antibody	Alexa Fluor 568, goat anti-mouse	1:500	Thermo Fisher Scientific Cat# A-11,004, RRID:AB_2,534,072
Primers			
	Target	**Forward/reverse primer (5′–3′)**	**Ta (°C)**	**Cycle number**
Pluripotency marker	Nanog	TGTGTTCTCTTCCACCCAGC/ ACCAGGTCTTCACCTGTTTGT	55	30
Pluripotency marker	Sox2	AGGGAGAGAAGTTTGAGCCC/ GCGAGGAAAATCAGGCGAAG	55	30
Pluripotency marker	Oct4	GACAGGGGGAGGGGAGGAGCTAGG/ CTTCCCTCCAACCAGTTGCCCCAAAC	66	30
Housekeeping gene	GAPDH	CATGTTCCAATATGATTCCACCC/ GGGATCTCGCTCCTGGAAGAT	57	40

3.7. Embryoid body formation

IPSC colonies were transferred to low attachment plates in differentiation medium (knockout DMEM, 20% FBS, 1% MEM NEAA, 2 mM GlutaMAX, and 0.1 mM beta-mercaptoethanol). After seven days EBs were reseeded onto gelatin-coated glass cover slips. EBs were allowed to differentiate for 10 days (at 37 °C/5% CO_2) and were fixed with 4% PFA for 15 min. Expression of nestin, muscle actin, and alpha-fetoprotein was proven accordingly to Peter et al. (2017).

3.8. STR analysis

DNA was extracted (Quick-gDNA Miniprep-Kit, Zymo Research) and quantified (PowerQuant™System, Promega). 250pg of DNA was amplified using a short tandem repeat assay, containing 16 STR loci and the Amelogenin gender-determining marker. PCR products were separated by multi-capillary electrophoresis. The fragment sizes were analyzed (GeneMapper ID-X v1.4 software, Applied Biosystems) and alleles were determined by comparison with an allelic ladder supplied with the PowerPlex® ESI 17 Pro-System or PowerPlex® ESX 17 Fast System (Promega).

3.9. Mycoplasma detection

PCR Mycoplasma Test Kit I/C (PK-CA91, PromoCell) was used following the manufacturer instructions to detect mycoplasma contamination (Suppl. Fig. 1A)

3.10. Targeted sequencing

Genomic DNA was isolated (NucleoSpin Tissue, Macherey&Nagel). Quantification was done using Qubit 2.0 fluorometer system (Thermo Fisher Scientific). Targeted sequencing library construction was performed using a custom designed Ion AmpliSeq™ NPC Panel (Thermo Fisher Scientific). 10 ng DNA per sample was used to amplify the complete coding sequence, according to the reference sequences annotated in human genome assembly hg19. Sequencing was performed on an Ion Torrent™ Personal Genome Machine™ System, using an Ion Torrent 318 V2 chip that allows minimal amplicon coverage of ≥ 4.619 reads per amplicon.

Variant calling and allele frequency determination was performed using Torrent Suite™ software and the variant caller plugin version 5.10.0.18 (Thermo Fisher Scientific) (Suppl. Fig.1 B,C).

3.11. One-Step RT-PCR

Total RNA was extracted using the Quick-RNA Miniprep kit (Zymo Research, R1054) according to the manufacturer's instruction. One-step Reverse Transcriptase PCR was performed using the OneStep RT-PCR kit (QIAGEN, 210,210) in an Eppendorf 5331 MasterCycler Gradient Thermal Cycler. 50 ng of total RNA was used for each reaction. The cycling times and the annealing temperatures were optimized for each primer pair. The PCR products were analysed on 2.5% agarose gel electrophoresis (Fig. 1O). Primer sequences are listed in Table 2.

Declaration of Competing Interest

None.

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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.2019.101606.

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