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Lab resource: Stem Cell Line

Generation of hiPSTZ16 (ISMMSi003-A) cell line from normal human foreskin fibroblasts

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ABSTRACT

Human foreskin fibroblasts from a commercial source were reprogrammed into induced pluripotent stem cells to establish a clonal stem cell line, hiPSTZ16 (ISMMSi003-A). These cells show a normal karyotype and full differentiation potential in teratoma assays. The described cells provide a useful resource in combination with other iPS cell lines generated from normal human foreskin fibroblasts to study source- and reprogramming method-independent effects in downstream applications.

Resource table

Unique stem cell line identifier	ISMMSi003-A
Alternative name(s) of stem cell line	hiPSTZ16 (ZCL1046)
Institution	Icahn School of Medicine at Mount Sinai
Contact information of distributor	Marion Dejosez Zwaka marion.dejosez@mssm.edu
Type of cell line	iPSC
Origin	Species: Human Age: Newborn Sex: Male Ethnicity: Not disclosed
Cell Source	Human foreskin fibroblasts CCD1079Sk (ATCC CRL2097)
Clonality	Clonal
Reprogramming method	Retroviral reprogramming with 5 factors (OCT4, SOX2, NANOG, KLF4, cMYC)
Associated disease	n/a
Disease associated locus	n/a
Known mutations or modification	n/a
Method of modification	n/a
Name of transgene and/or resistance	n/a
Inducible/constitutive system	n/a
Date archived/stock date	Sept. 23, 2010
Cell line repository/bank	n/a
Ethical approval	n/a

1. Resource details

Human foreskin fibroblast (HFF) cells (CCD1079Sk) were reprogrammed into the induced pluripotent stem (iPS) cell state through retroviral delivery (Takahashi et al. 2007, Lowry et al. 2008) of five reprogramming factors (OCT4, SOX2, NANOG, KLF4 and c-MYC). The established hiPSTZ16 (ISMMSi003-A) cells showed human embryonic stem cell-like morphology in phase contrast microscopy (Fig. 1A), and nuclear expression of the pluripotency marker OCT4 as detected by immunofluorescence (IF) staining (Fig. 1B). Additionally, flow cytometric (FC) analyses confirmed that more than 92% of cells were $OCT4^{+}/SSEA4^{+}$ double positive (Fig. 1C). The cells were karyotypically normal at passage 6 (Fig. 1D) and 16 (not shown) as determined by G-banding with a band resolution of 400–450. Furthermore, their short tandem repeat (STR) profile was identical to the one of their parental HFF cells (Fig. 1E). Finally, teratoma formation demonstrated the potential of hiPSTZ16 (ISMMSi003-A) cells to differentiate into cell types of all three germ layers (Fig. 1F) as we were able to detect ectoderm- (left), mesoderm- (middle) and endoderm-like (right) structures in H&E stained teratoma sections.

2. Materials and methods

2.1. Cell culture and reprogramming conditions

CCD1079Sk (CRL2097) HFF cells were cultured as recommended by the distributor (ATCC) and reprogrammed to the pluripotent state (Takahashi et al. 2007, Lowry et al. 2007) through retroviral delivery. Retroviral particles were produced in Phoenix-Ampho cells upon transfection of the plasmids pMXs-hOCT4, pMXs-hSOX2, pMXs-hcMYC, pMXs-hKLF4 and pMXs-hNANOG (Lowry et al. 2008; Addgene, Cat# 17964, 17965, 17966, 17967, and 18115, respectively). Emerging hiPS

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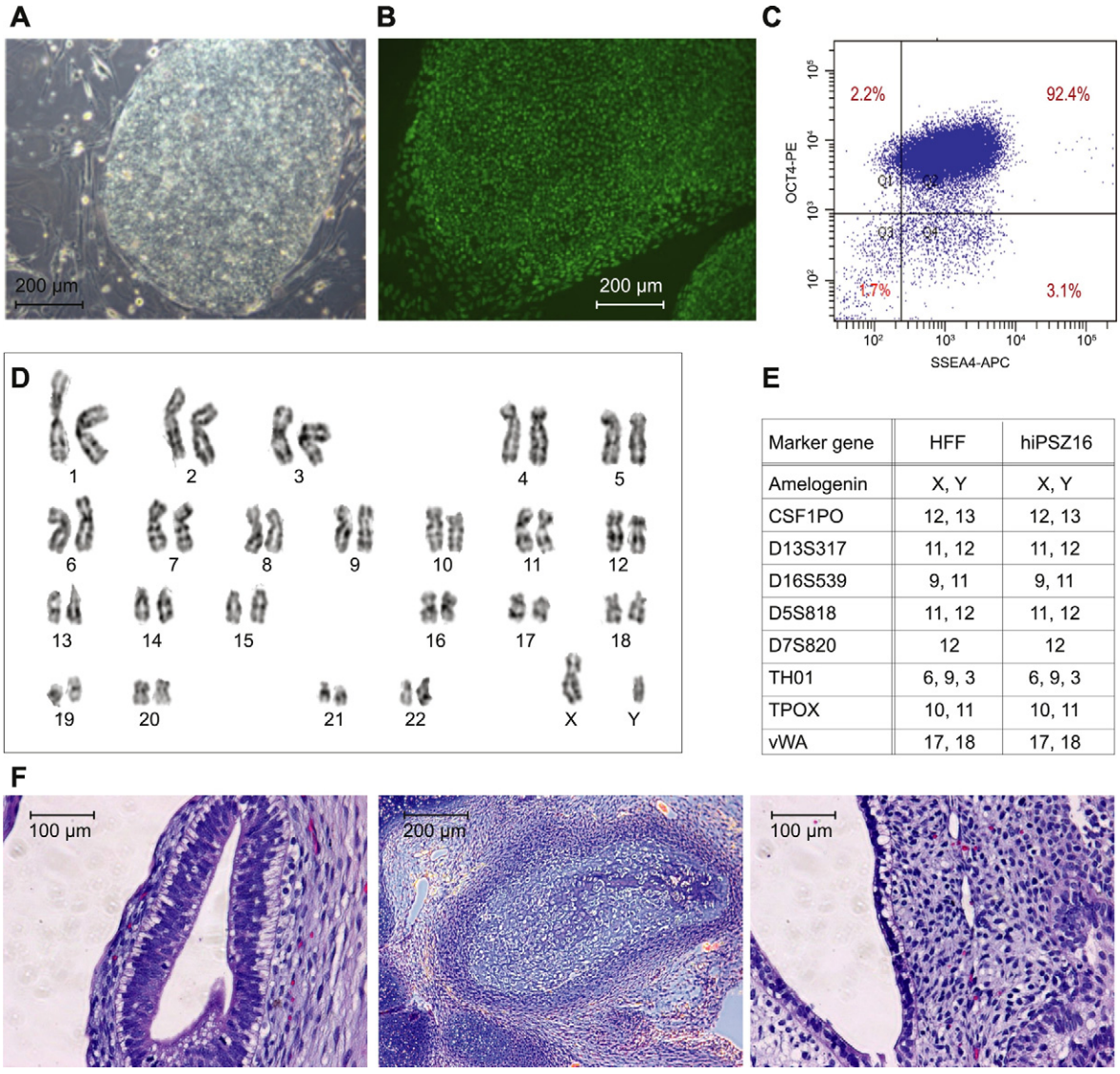

Marker gene	HFF	hiPSZ16
Amelogenin	X, Y	X, Y
CSF1PO	12, 13	12, 13
D13S317	11, 12	11, 12
D16S539	9, 11	9, 11
D5S818	11, 12	11, 12
D7S820	12	12
TH01	6, 9, 3	6, 9, 3
TPOX	10, 11	10, 11
vWA	17, 18	17, 18

Fig. 1. Characterization of hiPSC line hiPSTZ16 (ISMMSi003-A).

Table 1
Characterization and validation details.

Category	Test	Result	Data
Phenotype	Morphology	Colonies show ES cell-like morphology in phase contrast microscopy.	Fig. 1A
	Immunocytochemistry	Pluripotency marker OCT4 is expressed and shows nuclear localization.	Fig. 1B
	Flow cytometry	92.4% of cells are positive for OCT4 and SSEA4.	Fig. 1C
	RT-PCR	n/a	n/a
Genotype	Karyotype (G-banding)	G-banding at a resolution of 400–450 shows normal male karyotype (46XY).	Fig. 1D
	Blood group genotyping	n/a	n/a
	HLA tissue typing	n/a	n/a
	Sequencing	n/a	n/a
	Southern Blot	n/a	n/a
	WGS	n/a	n/a
Identity	STR analysis	9/9 sites matched	Fig. 1E
	Microsatellite PCR	n/a	n/a
Microbiology	Mycoplasma spp.	RT-PCR (h-IMPACT-III, Radil/Idexx) is negative for Mycoplasma spp.	With authors
	Virus screen	RT-PCR (h-IMPACT-III, Radil/Idexx) is negative for all viruses tested including HIV 1, HIV2, Hepatitis A, B, C, and Hantaan viruses (Hantaan, Seoul, Sin Nombre)	With authors
Differentiation	3 germ layer differentiation	Teratoma shows endoderm-, mesoderm- and ectoderm-like structures in histological sections.	Fig. 1F

Table 2
Antibody details.

Antibody description	Conjugate	Application	Dilution	Company	Catalog #	RRID ID
Mouse IgG3	APC	FC	1:5	R&D Systems	IC007A	AB_952035
Mouse IgG2B	PE	FC	1:6.6	R&D Systems	IC0041P	AB_357249
Normal Mouse IgG2B	n/a	IF	1:100	Santa Cruz Biotechnology	sc-3879	AB_737262
OCT4	PE	FC	1:6.6	R&D Systems	IC1759P	AB_416891
OCT4 (C10)	n/a	IF	1:100	Santa Cruz Biotechnology	sc-5279	AB_628051
SSEA4	APC	FC	1:5	R&D Systems	FAB1435A	AB_494994
Goat anti-Mouse IgG	AF488	IF	1:1000	Molecular Probes	A-11029	AB_138404

cell clones were expanded on mouse embryonic fibroblasts (Global Stem, GSC-6001G) as feeder layer in KO-DMEM supplemented with 20% KnockOut Serum Replacement, 1% Non-essential amino acids, 1% L-glutamine 0.1 mM, and 40 μg/ml β-FGF (Table 1).

2.2. Immunofluorescence staining

Cells were plated on matrigel, grown to the desired density, fixed in 2% formaldehyde in DPBS for 1 h, washed three times with DPBS, blocked in 1% sodium azide and 2% FBS in DPBS, and then incubated with the primary anti-OCT4 antibody (Table 2) or the corresponding isotype control (Table 2) diluted in blocking solution (1:100) for 30 min at RT. Cells were washed twice in DBPS, incubated in a 1:1000 dilution of the secondary goat anti-mouse-AF488 antibody (Table 2), washed as before, and nuclei were stained with 1 μg/ml DAPI in DPBS for 1 min.

2.3. Flow cytometric analysis

To determine the percentage of $OCT4^+/SSEA4^+$ cells, cells were harvested after a 10-min treatment with 0.05% trypsin. Approximately 0.5×10^6 cells were transferred to individual tubes, collected, and resuspended in 100 μl FACS buffer (2% FBS and 1% sodium azide in DPBS) containing 20 μl of the SSEA4-APC antibody or IgG3-APC isotype control (Table 2). Following a 30-minute incubation at 4 °C, cells were washed in DPBS, fixed in 2% formaldehyde for 30 min, washed, permeabilized in 0.1% saponine and 0.1% BSA in DBPS for 15 min, washed, and incubated in 100 μl FACS buffer containing 10 μl of the OCT4-PE antibody or IgG2B-PE isotype control (Table 2) for 1 h. The cells were then washed, resuspended in 300 μl DAPI-containing (1 μg/ml) FACS buffer and analyzed by flow cytometry (FACSAriaII).

2.4. STR, h-IMPACT analysis and G-banding

STR and h-Impact III test (including mycoplasm testing) were performed by Radil (Idexx). Karyotyping by G-banding was done by the Texas Children's Cancer Center Core (Houston, TX) at a band resolution of 400–450.

2.5. Teratoma formation assay

Cells were collected after collagenase treatment and approximately 2×10^6 cells in 100 μl PBS were injected into the hind leg muscle of SCID mice (Charles River strain 250). Teratoma were harvested after 8 weeks and fixed in 10% Formalin for 24 h. The fixed tissue was embedded in paraffin and 20 μm sections were stained with hematoxylin and eosin (H&E) by the Histology Core at Baylor College of Medicine (Houston, TX).

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