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

| Resource table | ISMMSi003-A |
|----------------|-------------|
| Unique stem cell line identifier | hiPSTZ16 (ZCL1046) |
| Alternative name(s) of stem cell line | n/a |
| 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 and c-MYC) |
| 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-hNANOG, pMXs-hKLF4 and pMXs-hMYC (Lowry et al. 2008; Addgene, Cat# 17964, 17965, 17966, 17967, and 18115, respectively). Emerging hiP
Fig. 1. Characterization of hiPSC line hiPSTZ16 (ISMMSi003-A).

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

**Table 1**
Characterization and validation details.

| 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  |
| vWFA        | 17, 18| 17, 18  |
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. Immuno-fluorescence 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 × 10⁶ 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 × 10⁶ 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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References

Lowry, W.E., Richter, L., Yachechko, R., Pyle, A.D., Tchieu, J., Sridharan, R., Clark, A.T., Plath, K., 2008. Generation of human induced pluripotent stem cells from dermal fibroblasts. Proc. Natl. Acad. Sci. U. S. A. 105 (8), 2883–2888.
Takahashi, K., Tanabe, K., Ohmukai, M., Narita, M., Ichisaka, T., Tomoda, K., Yamanaka, S., 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131 (5), 861–872.

| 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 |

Table 2
Antibody details.