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Four induced pluripotent stem cell lines (TRNDi021-C, TRNDi023-D, TRNDi024-D and TRNDi025-A) generated from fibroblasts of four healthy individuals

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Abstract

Expanded human skin fibroblast cells from four different aged healthy individuals, 11-year-old female, 1-year-old male, 2-month-old male, and 8-year-old male, were used to generate integration-free induced pluripotent stem cell (iPSC) lines TRNDi021-C, TRNDi023-D, TRNDi024-D, and TRNDi025-A, respectively, by exogenous expression of four reprogramming factors, human SXO2, OCT3/4, C-MYC, KLF4. The authenticity of established iPSC lines was confirmed by the expressions of stem cell markers, karyotype analysis, and teratoma formation. These iPSC lines could serve as young healthy controls for the studies involving patient-specific iPSCs.

1. Resource Table:

Unique stem cell lines identifier	TRNDi021-C
	TRNDi023-D
	TRNDi024-D
	TRNDi025-A
Alternative names of stem cell lines	HT725C (TRNDi021-C)HT727D
	(TRNDi023-D)HT728D
	(TRNDi024-D)HT729A
	(TRNDi025-A)

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scr.2020.102011>.

Institution	National Center for Advancing Translational Sciences
Contact information of distributor	wei.zheng@nih.gov
Type of cell lines	iPSC
Origin	Human
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	Integration-free Cytotune 2.0 SeV kit
Multiline rationale	Control iPSC lines from young healthy donors
Gene modification	NO
Type of modification	N/A
Associated disease	N/A
Gene/locus	N/A
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	4/25/2019
Cell line repository/bank	N/A
Ethical approval	NIGMS Informed Consent Form was obtained from four healthy individuals at time of sample submission.
	Confidentiality Certificate: CC-GM-15-004

2. Resource utility

These human iPSC lines could be a useful cell resource as young healthy controls for the studies involving patient-specific iPSCs and for research related to evaluations of compound toxicities.

3. Resource details

Accumulating evidences suggest that almost all types of diseased iPSCs can be generated, even in certain cases where reprogramming was previously thought to be very difficult or impossible. Most publications and cell resources are focused on the collection of patient-specific or disease-specific iPSC lines. However, there is still a shortage of control iPSC lines from of healthy, different-aged individuals. Here we report the generation of four human iPSC lines from skin fibroblasts of different-aged young healthy individuals. We have used a non-integrating viral vector containing human OCT3/4, KLF4, SOX2 and C-MYC transcription factors to transduce the fibroblasts according to the method described previously (Beers et al., 2012, 2015). All these four healthy iPSC lines demonstrated a classic embryonic stem cell morphology (Fig. 1A) and normal karyotypes (46, XY or 46, XX), as confirmed by G-banding karyotyping at passage 9 (Supplemental Fig. S1A), and exhibited positive expression of NANOG, TRA-1-60 and SSEA4, as measured by flow cytometry, and of OCT4A, NANOG, SOX2 and SSEA-4, as determined by immunofluorescence staining (Fig. 1B). Moreover, the pluripotency of these iPSCs lines was confirmed by a teratoma formation assay that showed their ability to differentiate into tissues of all three germ layers, including neural epithelium (N.E.) and pigment epithelium (P.E.)

from ectoderm, cartilage from mesoderm, and gut from endoderm, in vivo (Fig. 1C). Short tandem repeat (STR) DNA profiling analysis showed the genotypes of these four iPSC lines matched those of the parental fibroblasts (submitted in archive with journal). Mycoplasma tests in these iPSC lines showed the negative results (Supplemental Fig. S1B) (Table 2).

4. Materials and methods

4.1. Cell culture

Skin fibroblasts derived from healthy donors (GM02036 from 11-year female, GM05659 from 1-year male, GM05756 from 2-month male, and GM08398 from 8-year male) were obtained from Coriell Institute for Medical Research (Camden, NJ). Fibroblasts were cultured in DMEM (Life Technologies 11195–073) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin and 100 µg/ml streptomycin (Life technologies 10378–016) in a humidified incubator with 5% CO₂ and 20% O₂ at 37 °C. Cells were routinely trypsinized (0.05% trypsin/EDTA) and passaged until reprogramming. Human iPSCs were maintained on Matrigel (Corning, #354230) in the chemically-defined Essential 8 medium (Life Technologies, #A1517001) and passaged using 0.5 mM EDTA/DPBS, as described previously (Beers et al., 2012) (Table 1).

4.2. iPSC generation

Human iPSCs were established from skin fibroblasts using non-integrating Sendai virus technology as previously described (Takahashi and Yamanaka, 2006; Beers et al., 2012, 2015). Briefly, fibroblasts were infected according to the kit manual (#A16517, ThermoFisher). Infected cells were cultured in DMEM + 10%FBS + Pen/Strep medium for 4 days, before being re-plated onto Matrigel coated plates containing reprogramming medium and cultured until 25 days into reprogramming. Reprogramming medium is made by adding 64 mg/L L-ascorbic acid, 13.6 µg/L sodium selenite, 10 µg/mL Holo-transferrin, 100 ng/mL bFGF, 20 µg/mL insulin, 100 µM sodium butyrate to DMEM/F12 (Beers et al., 2015). Cells were switched to Essential 8 medium at day 20 after Sendai virus infection. Finally, iPSC colonies were picked based on human embryonic stem cell-like morphology, expanded, and cryopreserved by passage 3.

4.3. Immunocytochemistry

Immunofluorescence staining of iPSC colonies were performed as previously described (Lin et al., 2017). iPSCs were fixed in 2% paraformaldehyde (PFA) for 10 min at room temperature, washed with DPBS, and permeabilized with 0.2% Triton X-100/DPBS for 3 min. After blocking using 10 mg/ml bovine serum albumin (BSA) for 30 min, samples were incubated with primary antibody for SOX2, OCT4, NANOG, or SSEA4 for 1 h at room temperature. iPSCs were then washed with DPBS and incubated with Alexa 488 or 594-conjugated secondary antibodies for 1 h. Then nuclei staining was performed with 49,6-diamidino-2-phenylindole (DAPI). Cell images were captured using an EVOS FL cell imaging station (Thermo Fisher). The primary and secondary antibodies are listed in Table 3.

4.4. Flow Cytometry

iPSCs were suspended in permeabilization buffer (2% FBS and 0.2% Tween-20 in DPBS) for 10 min at room temperature and then incubated with the fluorophore-conjugated antibodies for 1 h at 4 °C. Nonspecific rabbit or mouse IgG served as the negative control (Antibodies used are listed in Table 3). After washing, the cells were detected, and the fluorescence signals were determined using a FACScan flow cytometer (BD Biosciences) at 488 nm. At least 10,000 events were collected from the cell gate. The data were then analyzed on a BD Accuri™ C6 Flow-Cytometry system (BD Biosciences).

4.5. G-banding karyotyping

G-banding karyotyping was performed by WiCell Cytogenetics lab (Madison, WI) using twenty randomly selected metaphases.

4.6. Short tandem repeat (STR) analysis

STR analysis for TRNDi021-C, TRNDi023-D, and GM5659 fibroblast was performed by Johns Hopkins University GRCF DNA Services using PowerPlex 18D System (Promega). STR analysis for GM2036 fibroblast, GM5756 fibroblast, GM8398 fibroblast, TRNDi024-D, and TRNDi025-A was performed by WiCell Cytogenetics lab using Powerplex 16 System (Promega). Genomic DNA used in STR analysis was extracted from the fibroblasts and iPSCs using DNeasy Blood and Tissue Kit (Qiagen).

4.7. Mycoplasma detection

Mycoplasma testing were performed following MycoAlert kit instructions (Lonza). By measuring the level of ATP in an iPSC line both before (read A) and after the addition of the MycoAlert substrate (read B), a ratio can be obtained which is indicative of the presence or absence of mycoplasma. Ratio B/A > 1.2 indicates mycoplasma positive and Ratio B/A < 0.9 indicates mycoplasma negative.

4.8. Teratoma formation assay

Human iPSCs were collected after 0.5 mM EDTA/DPBS treatment and subcutaneously injected into NSG mice (JAX #005557) with cold Matrigel (Corning #354277). Visible tumors typically appeared 6–8 weeks post injection. Palpable tumors were removed and fixed in 10% Neutral Buffer Formalin. They were then embedded in paraffin and stained with H&E according to standard procedures to check their differentiation capacity into all three germ layers in vivo.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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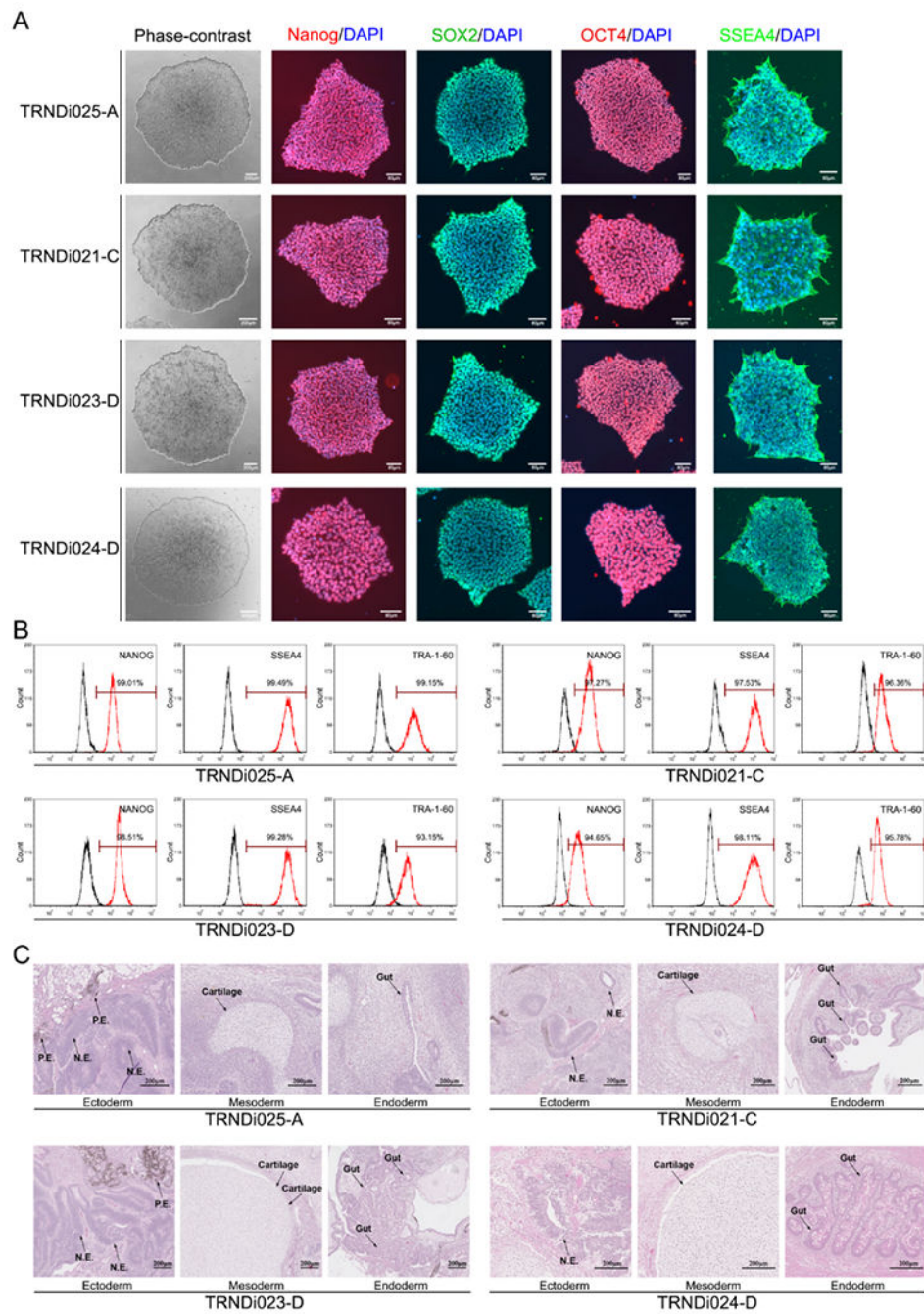


Fig. 1. (A) Images of phase contrast microscopy and immunofluorescence staining of pluripotency markers of 4 iPSC lines. (B) Flow cytometry analysis of pluripotency markers of 4 iPSC lines. (C) Teratoma formation assay shows all 4 iPSC lines can generate three germ layers, including neural epithelium (N.E.) and pigment epithelium (P.E.) from ectoderm, cartilage from mesoderm, and gut from endoderm, in vivo.

Table 1

Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
TRNDi021-C	TRNDi021-C	Female	11-year-old	Caucasian	Wild-type	N/A
TRNDi023-D	TRNDi023-D	Male	1-year-old	Caucasian	Wild-type	N/A
TRNDi024-D	TRNDi024-D	Male	2-month-old	Caucasian	Wild-type	N/A
TRNDi025-A	TRNDi025-A	Male	8-year-old	Caucasian	Wild-type	N/A

Table 2

Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1A
Phenotype	SOX2, OCT3/4, NANOG, SSEA-4 TRNDi021-C (TRA-1-60: 96.36% SSEA-4: 97.53% NANOG: 97.27%)		
	Qualitative analysis: Immunocytochemistry Quantitative analysis: Flow Cytometry	TRNDi023-D (TRA-1-60: 93.15% SSEA-4: 99.28% NANOG: 98.51%) TRNDi024-D (TRA-1-60: 95.78% SSEA-4: 98.11% NANOG: 94.65%), TRNDi025-A (TRA-1-60: 99.15% SSEA-4: 99.49% NANOG: 99.01%)	Fig. 1A Fig. 1B
Genotype	Karyotype (G-banding) and resolution	46,XY or 46, XX Resolution: 425-475	Supplemental Fig. S1A
Identity	Microsatellite PCR (mPCR) OR STR analysis	not performed	N/A
Mutation analysis (IF APPLICABLE)	STR analysis	16 or 18 sites tested, all sites matched	Submitted in archive with journal
	Sequencing Southern Blot OR WGS	N/A N/A	N/A N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. Negative	Supplementary Fig. S1B
Differentiation potential	e.g. Embryoid body formation OR Teratoma formation OR Scorecard OR Directed differentiation	Teratoma formed with tissues of all three germ layers (Ectoderm, Mesoderm, Endoderm) in vivo	Fig. 1C
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping HLA tissue typing	N/A N/A	N/A N/A

Table 3

Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Mouse anti-SOX2	1:50	R & D systems, Cat# MAB2018, RRID: AB_358009
Pluripotency Markers	Rabbit anti-NANOG	1:400	Cell signaling, Cat# 4903, RRID: AB_10559205
Pluripotency Markers	Rabbit anti-OCT4	1:400	Thermo Fisher, Cat# 701756, RRID: AB_2633031
Pluripotency Markers	Mouse anti-SSEA4	1:1000	Cell signaling, Cat# 4755, RRID: AB_1264259
Secondary Antibodies	Donkey anti-Mouse IgG (Alexa Fluor 488)	1:400	Thermo Fisher, Cat# A21202, RRID: AB_141607
Secondary Antibodies	Donkey anti-Rabbit IgG (Alexa Fluor 594)	1:400	Thermo Fisher, Cat# A21207, RRID: AB_141637
Flow Cytometry Antibodies	Anti-Tra-1-60-DyLight 488	1:50	Thermo Fisher, Cat# MA1-023-D488X, RRID: AB_2536700
Flow Cytometry Antibodies	Anti-Nanog-Alexa Fluor 488	1:50	Millipore, Cat# FCABS352A4, RRID: AB_10807973
Flow Cytometry Antibodies	anti-SSEA-4-Alexa Fluor 488	1:50	Thermo Fisher, Cat# 53-8843-41, RRID: AB_10597752
Flow Cytometry Antibodies	Mouse-IgM-DyLight 488	1:50	Thermo Fisher, Cat# MA1-194-D488, RRID: AB_2536969
Flow Cytometry Antibodies	Rabbit IgG-Alexa Fluor 488	1:50	Cell Signaling, Cat# 4340S, RRID: AB_10694568
Flow Cytometry Antibodies	Mouse IgG3-FITC	1:50	Thermo Fisher, Cat# 11-4742-42, RRID: AB_2043894