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Lab Resource: Multiple Stem Cell Lines

Generation of nine induced pluripotent stem cell lines as an ethnic diversity panel

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A B S T R A C T

Human induced pluripotent stem cells (iPSCs) provide a potentially unlimited source of differentiated cells from individuals with specific genetic backgrounds. Using self-replicative RNA reprogramming technology, we generated nine iPSC lines from endothelial progenitor cells (EPCs) derived from blood samples of three different ethnicities: Black or African American, Latino or Hispanic, and Non-Hispanic White. The resulting iPSC lines showed normal karyotype in large part, expressed pluripotency marker genes, and spontaneously differentiated *in vitro* into the three germ layers. These iPSC lines offer the potential to generate tissues with ethnic diversity, and thus afford a valuable tool for ethnic-related toxicological applications.

Resource table.

Unique stem cell lines identifier	OARSAi001-A OARSAi002-A OARSAi003-A OARSAi004-A OARSAi005-A OARSAi006-A OARSAi007-A OARSAi008-A OARSAi009-A
Alternative names of stem cell lines	S04 (OARSAi001-A) S06 (OARSAi002-A) S09 (OARSAi003-A) S10 (OARSAi004-A) S11 (OARSAi005-A) S12 (OARSAi006-A) S14 (OARSAi007-A) S18 (OARSAi008-A) S20 (OARSAi009-A)
Institution	Office of Applied Research and Safety Assessment, CFSAN, U.S. FDA (OARSA)
Contact information of distributor	Xiugong Gao, xiugong.gao@fda.hhs.gov
Type of cell lines	iPSC
Origin	Human
Cell Source	Blood
Clonality	Clonal or mixed
Method of reprogramming	Self-replicative RNA (srRNA) reprogramming with 4 factors (Oct4, Sox2, Klf4, c-Myc)
Multiline rationale	A panel of non-diseased lines with diverse ethnicities
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	N/A
Cell line repository/ bank	N/A
Ethical approval	Whole blood collection from healthy donors was conducted by AllCells with informed consent under the Protocol #7000-SOP-049 Version 2.0 approved by Alpha Independent Review Board (Alpha IRB)

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1. Resource utility

Ethnic diversity in toxic response to chemicals has been reported (Yasuda et al. 2008). iPSCs afford a well-defined source for deriving tissue-specific cells that can provide physiologically relevant *in vitro* models (Suter-Dick et al. 2015). The iPSC lines described here could be used as a panel for ethnic-related toxicological applications.

2. Resource details

Mononuclear cells (MNCs) from umbilical cord blood or adult peripheral blood of healthy individuals with different ethnicities were used as the starting materials (Table 1). iPSCs were generated from MNCs following a two-step protocol in which endothelial progenitor cells (EPCs) were first derived from MNCs under endothelial-selective conditions, and then reprogrammed into iPSCs using a self-replicative RNA (srRNA) (Gao et al. 2017). The srRNA was constructed from a self-replicating Venezuelan equine encephalitis (VEE) virus RNA replicon encoding open reading frames (ORFs) of the four reprogramming factors OCT4, KLF4, SOX2, and c-MYC (Yoshioka et al. 2013). The iPSC lines were isolated from individual colonies mechanically picked up from the reprogramming plates, and were continuously expanded to passage 15. Endpoint RT-PCR (in the nsP4 region) showed no amplification of VEE RNA in all the lines (Supplementary Fig. 1A), indicating complete clearance of srRNA from the cells. G-banding karyotyping revealed that 6 of the 9 lines had normal diploid 46, XY karyotype with no detectable abnormalities, with the other 3 having abnormalities in chromosome 8 or 10 in some of the cells examined (Supplementary Fig. 1B). It is worth to note that for toxicological applications, even large chromosomal aberrations such as aneuploidy are permissive for iPSC-derived hepatocytes (Noto et al. 2014). Human pathogen screening indicated that all the lines are negative for bacteria, fungi, mycoplasma, and viruses (Supplementary Table 1).

Pluripotency of the iPSC lines was confirmed by expression of the pluripotency markers SSEA4, TRA-1-60, OCT4, and SOX2, as revealed by immunofluorescence staining (Fig. 1A). Differentiation potential of the lines was verified by *in vitro* spontaneous differentiation through embryoid body (EB) formation towards the three germ layers, as revealed by immunofluorescence detection of ectodermal markers paired box protein 6 (PAX6) and SRY-Box 1 (SOX1), mesodermal markers brachyury and alpha smooth muscle actin (α-SMA), as well as endodermal markers alpha-fetoprotein (AFP) and SRY-box 17 (SOX17) (Fig. 1B). The identity of the iPSC lines was confirmed by short tandem repeat (STR) analysis, which matched to that of the original MNCs. The complete characterization is summarized in Table 2.

3. Materials and methods

3.1. iPSC generation and culture

iPSCs were generated from endothelial progenitor cells (EPCs) derived from blood mononuclear cells (MNCs) using the StemRNA-SR Reprogramming Kit from Stemgent (Cambridge, MA) following the manufacturer's protocol. Details for the derivation and reprogramming of EPCs have been reported elsewhere (Gao et al. 2017). iPSC colonies were cultured in NutriStem XF/FF medium (Stemgent) during reprogramming and the first two clump passages. Starting from passage 3, iPSCs were transitioned to Cellartis DEF-CS Culture System (Takara Bio USA, Mountain View, CA), which supports single cell passage and culture of iPSCs in a feeder-free and defined environment. For single cell dissociation, iPSCs were washed once with phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+} (PBS −/−; Gibco, Gaithersburg, MD) and treated with TrypLE Select 1× (Gibco) for 5 min at 37 °C followed with pipetting to ensure single cell dissociation. The single cell suspension was then centrifuged at 200*g* for 3 min, resuspended in DEF-CS medium, and seeded into 6-well cell culture plate coated with COAT-1 (Takara Bio USA) at a density of 4.0–5.0 × 10^4 cells/cm^2. Cells were maintained at 37 °C, 5% CO_2, and > 90% humidity, and culture medium was changed every day, until the cells reach a confluence of 1.5–3.0 × 10^5 cells/cm^2, which normally occurred 3–4 days post passage. With each passage cells were re-dissociated into single cells and transferred to a new tissue culture plate coated with COAT-1.

3.2. Immunofluorescence staining

Cells were fixed using 4% (v/v) paraformaldehyde (Alfa Aesar; Tewksbury, MA), washed three times with PBS containing 0.2% (v/v) Tween (PBST) (Fisher Scientific; Waltham, MA) and permeabilized using 0.15% (v/v) TritonX-100 (Sigma-Aldrich; St. Louis, MO) in PBS for 1 h at 25 °C. After permeabilization, cells were blocked with 1% (v/v) bovine serum albumin (BSA) (Invitrogen; Carlsbad, CA) in PBST (PBSTB) for 30 min at 25 °C. After gentle removal of PBSTB, cells were incubated with primary antibody (Table 3) in PBSTB overnight at 4 °C. After the overnight incubation, cells were washed three times with PBST and stained with secondary antibody (Table 3) in PBSTB for 1 h at 25 °C. Then cells were washed three times in PBST and stained with Hoechst dye (Invitrogen). Images of the stained cells were taken under Leica DMi8 fluorescence microscope with CCD camera.

3.3. Induction of differentiation

The iPSCs were subjected to suspension culture in EB maintenance media (iXCells Biotechnologies; San Diego, CA) to form EBs. The EBs were maintained in suspension culture for 1–2 weeks with daily media change and attached to gelatin-coated dishes in the same media for another 1–2 weeks before antibody staining.

3.4. Karyotype analysis

Cytogenetic analysis was performed on 20 G-banded metaphase cells per line by iXCells Biotechnologies.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.07.013.

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity[a]	Genotype of locus	Disease
S04 (OARSAi001-A)	S04	Male	0	NHW	N/A	No
S06 (OARSAi002-A)	S06	Male	0	NHW	N/A	No
S09 (OARSAi003-A)	S09	Male	0	BAA	N/A	No
S10 (OARSAi004-A)	S10	Male	0	BAA	N/A	No
S11 (OARSAi005-A)	S11	Male	0	NHW	N/A	No
S12 (OARSAi006-A)	S12	Male	0	NHW	N/A	No
S14 (OARSAi007-A)	S14	Male	23	LOH	N/A	No
S18 (OARSAi008-A)	S18	Male	27	LOH	N/A	No
S20 (OARSAi009-A)	S20	Male	0	NHW	N/A	No

[a] BAA, Black or African American; LOH, Latino or Hispanic; NHW, Non-Hispanic White.