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Lab resource

Integration-free T cell-derived human induced pluripotent stem cells (iPSCs) from a healthy individual: WT-iPSC4

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ABSTRACT

Expanded human T cells from a Japanese healthy male were used to generate integration-free induced pluripotent stem cells (iPSCs) by exogenous expression of four reprogramming factors, *OCT3/4*, *SOX2*, *cMYC*, *KLF4*, using Sendai virus vector (SeVdp). The authenticity of established iPSC line, WT-iPSC4, was confirmed by the expressions of stem cell markers and the differentiation capability into three germ layer. WT-iPSC4 may be a useful cell resource as a normal control for the comparative study using disease-specific iPSCs.

Resource table:

Name of Stem Cell line	WT-iPSC4
Institution	Department of Dermatology, The Jikei University School of Medicine
Person who created resource	Shiho Kawagoe, Munenari Itoh
Contact person and email	Munenari Itoh: seafowl@jikei.ac.jp
Date archived/stock date	Aug 26, 2014
Origin	human T cells
Type of resource	Biological reagent: human induced pluripotent stem cell (iPS); derived from a Japanese healthy male
Sub-type	Induced pluripotent stem cells (iPSCs)
Key transcription factors	*OCT3/4*, *SOX2*, *cMYC*, *KLF4*
Authentication	Identity and purity of cell line confirmed as shown in Fig. 1
Link to related literature	N/A
Information in public databases	N/A
Ethics	Institutional ethics committee approval obtained (No. 23-271(6732))/Patient written informed consent obtained

1. Resource details

Expanded T cells isolated from a 38 year-old Japanese healthy male were reprogrammed employing Sendai virus vectors (SeVdp) expressing four reprogramming factors, *OCT3/4*, *SOX2*, *cMYC*, *KLF4*. SeVdp is integration-free vector, and the absence of reprogramming genes in established iPSC line, WT-iPSC4, was confirmed by PCR analysis (Fig. 1A). DNA fingerprint analysis of WT-iPSC4 and expanded T cells provided the origin of cell source (Fig. 1B). The authenticity of WT-iPSC4 was confirmed by the followings; (1) the expression of stem cell markers by immunostaining (Fig. 1C) and RT-PCR (Fig. 1D), (2) the differentiation capability into three germ layers using in vitro differentiation through embryoid bodies (EBs) and teratoma formation (Fig. 1E). In addition, WT-iPSC4 maintains normal karyotype (46, XY) (Fig. 1F), and Bisulfite sequencing revealed that the *NANOG* promoter region in WT-iPSC4 was unmethylated (Fig. 1G).

[1] Equally contributed.

2. Materials and methods

2.1. Cell culture

Monocytes were isolated from a volunteer's peripheral blood sample using SepMate-50 (STEMCELL technologies, Inc., Canada) and Ficoll-Paque Plus (GE Healthcare). Consequently, T cell was expanded in TLY culture kit (LYMPHOTEC, Inc., Japan) for 5–10 days at 37 °C at 5% CO_2.

2.2. iPSC generation.

Expanded T cells were reprogrammed by SeVdp as previously described (Nishimura et al., 2011) and (Itoh et al., 2016). Briefly, the transfected cells were seeded on feeder cell layer in RPMI1640 with 10% fetal bovine serum and interleukin-2 (PeproTech). After 2 days, the medium was changed to Primate ES cell culture medium (ReproCELL Inc., Japan) supplemented with basic fibroblast growth factor (bFGF) (5 ng/ml, R&D Systems), and cultured until colonies appeared. Established iPSCs were maintained in human iPS cell medium (Itoh et al., 2016). To completely remove SeVdp, siRNA mixture (Itoh et al., 2016) was added several times into the medium.

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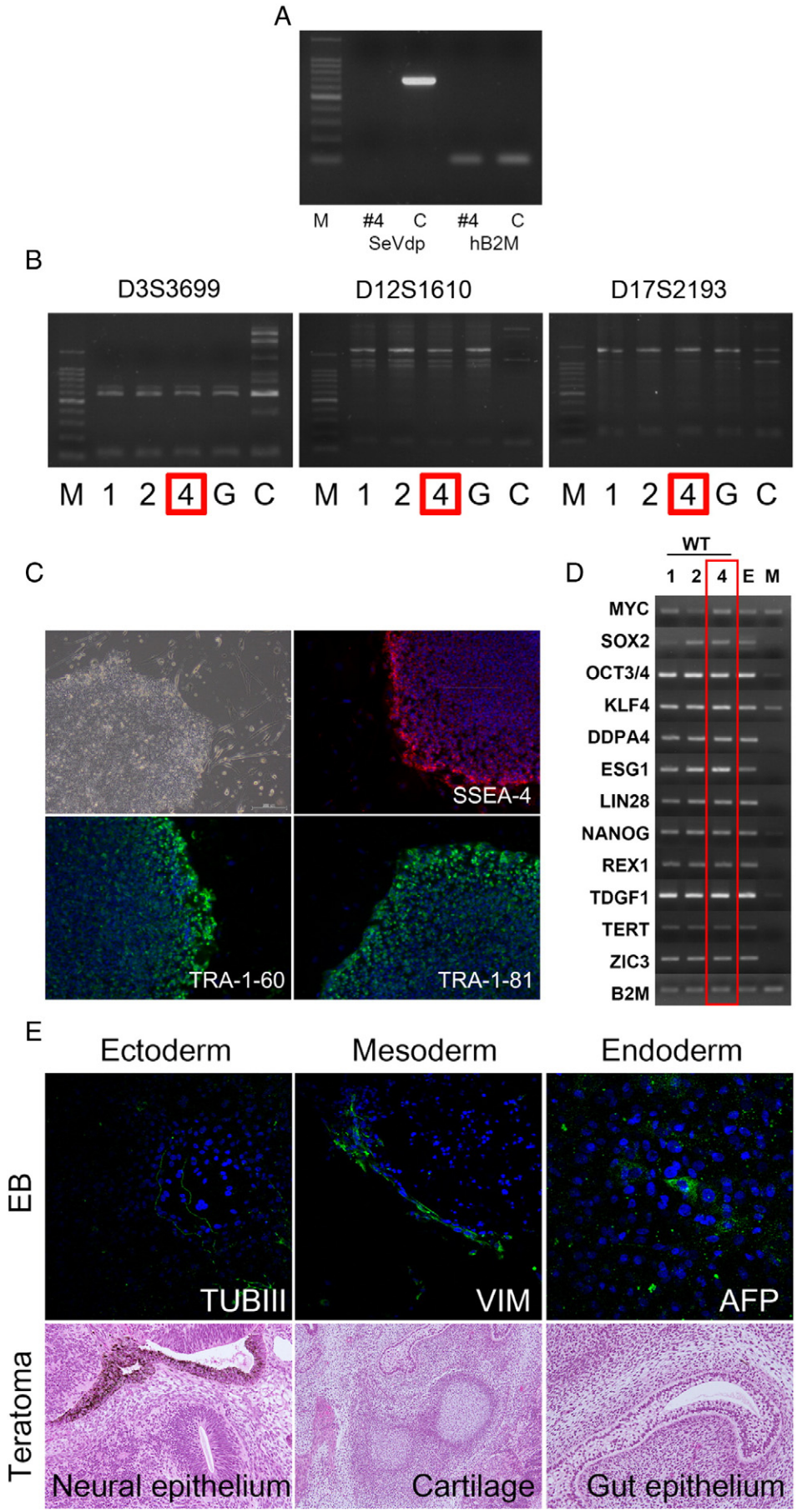
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B
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C
SSEA-4
TRA-1-60
TRA-1-81
D
WT
1 2 4 E M
MYC
SOX2
OCT3/4
KLF4
DDPA4
ESG1
LIN28
NANOG
REX1
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EB
TUBIII VIM AFP
Teratoma
Neural epithelium Cartilage Gut epithelium

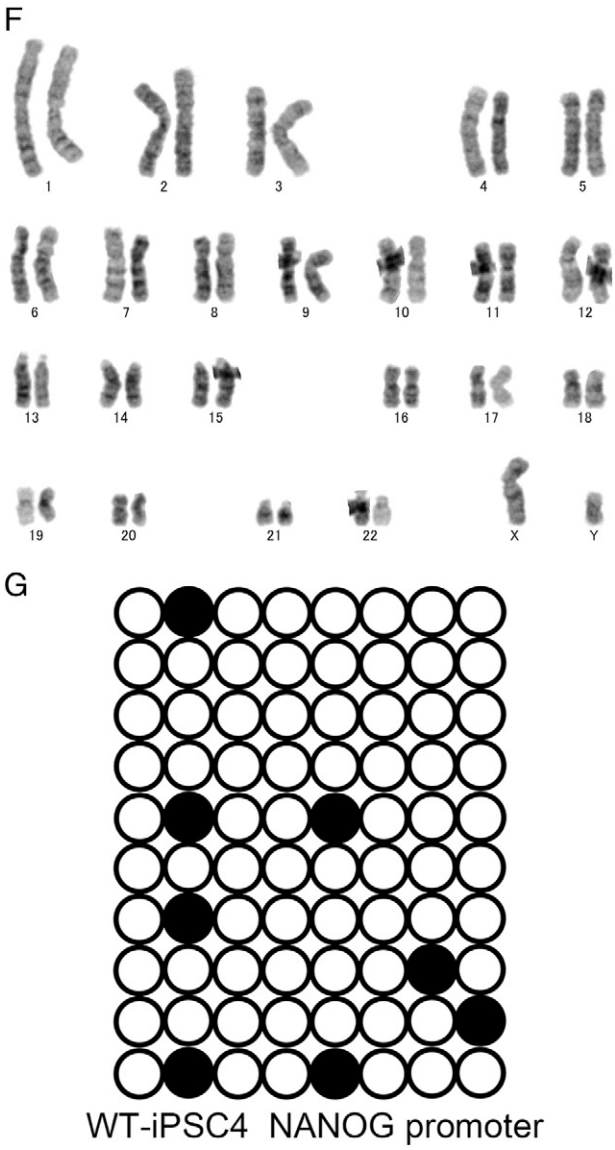

Fig. 1. Characterization of WT-iPSC4. (A) Confirmation of the absent of SeVdp-related reprogramming gene by RT-PCR (M: marker, #1: WT-iPSC4, C: control transfected cell sample). (B) DNA fingerprint analysis by RT-PCR identified the origin of WT-iPSC4 (M: marker, Red square: WT-iPSC4 genomic DNA, G: genomic DNA isolated from original T cells, C: genomic DNA isolated from a different indiviual). The expression of stem cell markers in WT-iPSC4 was detected by immunostaining (C) and RT-PCR (D) (Red square: WT-iPSC4 cDNA, E: embryonic stem cell cDNA, M: monocyte cDNA). (E) Embryoid body and teratoma formation indicated the differentiation capability into three germ layer, ectoderm (βIII-tubulin and neural epithelium), mesoderm (vimentin and cartilage) and endoderm (α-fetoprotein and gut epithelium). (F) Karyotype was normal (46, XY). (G) The NANOG promoter region in WT-iPSC4 was almost completely unmethylated (White circle: unmethylated CpGs, black circle: methylated CpGs). All the data suggested the authenticity of WT-iPSC4.

2.2. DNA fingerprint analysis

DNA fingerprint analysis was performed by PCR amplification with specific primers supplied in (Itoh et al., 2011).

2.3. RT-PCR and PCR

RNA was extracted using an RNeasy Mini Kit (Qiagen), and complementary DNA was synthesized using 2 μg RNA by SuperScript III reverse transcriptase and Oligo-dT primer (Invitrogen) according to the manufacturer's instructions. PCR reactions were performed with Platinum PCR SuperMix (Invitrogen). All primer sequences are supplied in (Itoh et al., 2011) and (Itoh et al., 2016).

2.4. Immunostaining

iPSCs and EBs were fixed with 4% paraformaldehyde(PFA)/PBS and permeabilized with 0.1% Triton-×100/PBS. After blocking using 10% goat sera/PBS, samples were incubated for 1 h at room temperature (RT) or overnight at 4 °C with primary antibodies. After three rinses with PBS, incubation with appropriate secondary antibodies was performed for 1 h at RT. Nuclei staining was performed with Hoechst33342 (1:1000, Fisher Scientific)/PBS. Conforcal microscopy (OLYMPUS FV-300) was used to visualize and capture the immunostained cells with good resolution. All antibodies are supplied in (Itoh et al., 2016).

2.5. Karyotyping analysis

Karyotyping analysis was offered to Nihon Gene Research Laboratories, Inc., Japan.

2.6. Methylation assay

A total of 1 μg genomic DNA extracted from WT-iPSC4 was treated with EZ DNA Methylation-Gold Kit (ZYMO Research, Corp.) for bisulfite reaction. The promoter region of the *NANOG* gene was amplified by PCR using gene-specific primers (Itoh et al., 2011). PCR product was subcloned into pCR2.1 vector (Invitrogen), and 10 clones were sequenced for defining methylation status of the *NANOG* promoter region.

2.7. In vitro differentiation

EBs were made in hiPSCM without bFGF as previously described in (Itoh et al., 2011) For enhancing spontaneous differentiation, EBs were attached on gelatin-coated chamber slides and incubated in DMEM with 10% FBS for 14 days before immunostaining.

2.8. Teratoma formation

Colonies of WT-iPSC4 were collected by 1 mg/ml collagenase IV (Invitrogen) treatment and subcutaneously injected into nude mice (CLEA Japan, Inc.) with Matrigel (Corning, Inc.). Palpable tumors typically appeared 1–2 months after injection. Tumors were collected and processed for H&E staining according to standard procedures to check their differentiation capacity into all three germ layers in vivo.

Acknowledgements

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