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Lab Resource: Stem Cell Line

Generation of Hermansky–Pudlak Syndrome Type 1 (HPS1) induced pluripotent stem cells (iPSCs)

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ABSTRACT

Hermansky–Pudlak syndrome (HPS) is a rare autosomal recessive disorder characterized by deficiencies in lysosome-related organelles such as melanosomes and platelet-dense granules. The disorder is classified into nine different subtypes (HPS1–HPS9) based on genetic mutations in 9 unique genes. Here we describe the generation of an HPS1 iPSC line (*CHOPHPS1*) using a Cre-excisable polycistronic STEMCCA lentivirus. This line was derived from human fibroblasts isolated from a patient carrying a duplicative mutation in the HPS1 gene. The patient presented with oculocutaneous albinism, early pulmonary fibrosis, and hemorrhagic diathesis.

Resource table: *CHOPHPS1*

Name of stem cell construct	CHOPHPS1
Institution	The Children's Hospital of Philadelphia (CHOP)
Person who created resource	Lin Lu
Contact person and email	Deborah L. French, frenchd@email.chop.edu
Date archived/stock date	2012
Origin	Human skin fibroblasts (GM14609, Coriell Cell Repositories)
Type of resource	Biological reagent: iPSC derived from human skin fibroblasts
Subtype	Cell Line
Key transcription factors	Oct4, Sox2, Klf4, c-Myc
Authentication	Identity and purity of cell line confirmed (Fig. 1)
Link to related literature	Oh et al., 1996
Information in public databases	N/A

Resource details

Human skin fibroblasts isolated from a 26 year old Puerto Rican female HPS1 patient were obtained from the Coriell Cell Repositories (Camden, NJ). The parental line, GM14609, carries a homozygous 16 bp duplication (codons 491–496) in exon 15 of the HPS1 gene. The cells were transduced with STEMCCA Cre-excisable constitutive polycistronic lentivirus expressing Oct4, Klf4, Sox2, and c-myc. Cre-mediated excision of the vector following reprogramming was demonstrated (Fig. 1A). The mutation was validated by DNA sequencing and quantitative PCR (qPCR) (Fig. 1B). The cells exhibited a normal karyotype (46, XX) upon G-band analysis (Fig. 1C).

Pluripotency was verified by gene expression of stem cell markers DMNT3B, REX1, ABCG2, OCT4, NANOG, and SOX2 using qPCR (Fig. 1E). In addition, expression of pluripotent surface markers at single cell resolution was confirmed by flow cytometry (Fig. 1D). Differentiation capacity into three germ layers was confirmed by *in vivo* teratoma formation (Fig. 1F).

Materials and methods

Cell culture and iPSC reprogramming

Human HPS1 fibroblast line, GM14609, was cultured in IMDM (Mediatech) containing 10% FBS at 37 °C and 5% CO_2 in a humidified incubator. STEMCCA lentiviral vector was produced, and cells were infected as previously described (Somers et al., 2010). The STEMCCA Cre-Excisable Constitutive Polycistronic viral vector used for reprogramming encoded Oct4, Klf4, Sox2, and c-myc. Human fibroblasts were incubated with (5–10 μl) STEMCCA virus plus 5 μg/ml of polybrene in fibroblast medium. Six days after infection, cells were replated on 100-mm dishes containing irradiated mouse embryonic fibroblasts (MEFs). The cells were maintained in IMDM with 10% FBS, NEAA (1%), glutamine (1%), penicillin/streptomycin (1%), 50 μg/ml ascorbic acid, and 4 ng/ml bFGF for 4 days. Thereafter, cells were maintained in human embryonic stem cell medium (HES) containing 4 ng/ml of bFGF. The medium was replenished every 2–3 days for

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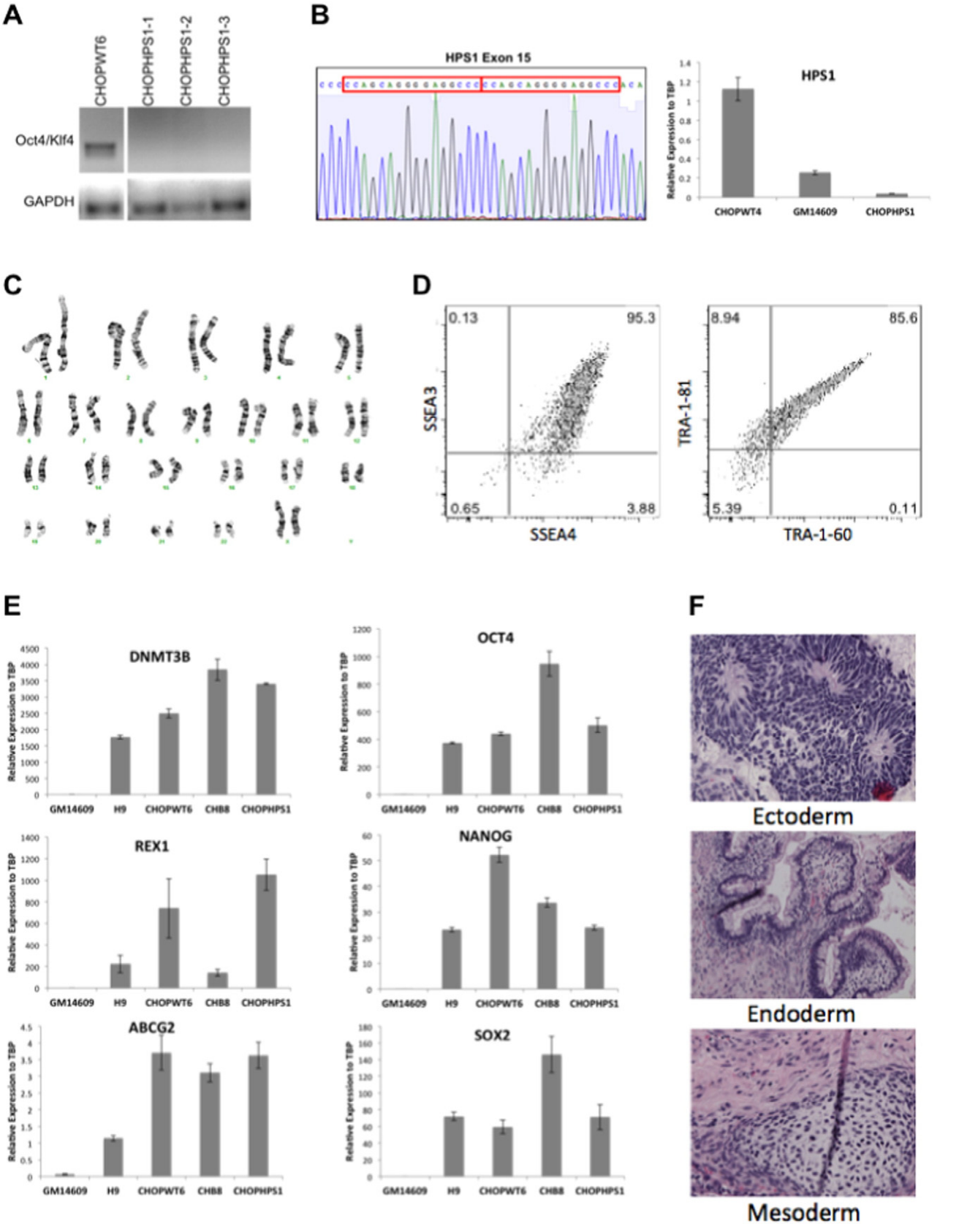

Fig. 1. Generation and Characterization of CHOPHPS1. (A) Cre-mediated excision of the lentiviral vector in three clones of the CHOPHPS1 iPSC line. Cells from a non-excised lentiviral iPSC line were used as a positive control. (B) Validation of the HPS1 mutation by DNA sequencing of HPS1 exon 15 and qPCR measurement of the HPS1 gene expression. (C) Karyotype analysis of CHOPHPS1. (D) Flow cytometry profiles of surface markers on undifferentiated cells: SSEA3, SSEA4, TRA-1-60, and TRA-1-81. (E) Quantitative PCR of relative gene expression of pluripotency genes compared to embryonic stem cells (H9 and CHB8), a control iPSC line (CHOPWT6), and the parental fibroblast line (GM14609). (F) H&E staining of teratoma sections identifying the three germ layers.

3 weeks until uniform colonies were generated. The iPSC colonies were mechanically isolated and expanded on MEFs.

Cre-mediated STEMCCA excision

Transgene removal was performed as previously described (Somers et al., 2010). Briefly, iPSCs were transfected with 2 μg/well of pHAGE2-Cre-IRES-PuroR plasmid DNA using Hela Monster transfection reagent (Mirus, Madison, Wi) according to the manufacturer's instructions. Cre-excised iPSC colonies were isolated by growth in puromycin (1.2 μg/ml) selection medium 24 h post-transfection for 48 h. Genomic DNA was isolated using the DNeasy Blood & Tissue kit (Qiagen, Valencia, CA) from each subclone after 2–3 weeks in culture and screened for hSTEMCCA transgenes using the following primers: OCT4-Tg-F: 5′GGT GCG CCA GTA AAG CAG ACA TTA AAC3′; KLF4-Tg-R: $^{5'}$CAG ACG CGA ACG TGG AGA AAG A$^{3'}$ and GAPDH-F: $^{5'}$GTG GAC CTG ACC TGC CGT CT$^{3'}$; GAPDH-R: $^{5'}$GGA GGA GTG GGT GTC GCT GT$^{3'}$.

Mutation verification

PCR amplification was performed on genomic DNA isolated from iPSCs using the referenced primer set: $^{5'}$GATGGTCCACAAAGGACGAG$^{3'}$ and $^{5'}$GCGTGAAGGAAGTACGGGCC$^{3'}$ (Oh et al., 1996). Products were sequenced by the CHOP NAPCore facility. Expression of the mutated HPS1 gene was shown by qPCR using the following primers: $^{5'}$ATAG CAGAGGACACCCTCCAAACA$^{3'}$ and $^{5'}$GGCAGTAGCTTTCCTTCACGTTGG$^{3'}$.

Karyotype analysis

Chromosomal G-band analyses were performed in the Genomics Core Laboratory (CHOP).

Flow cytometry analysis

Expression of pluripotency markers was evaluated by flow cytometry using the following antibodies: Alexa-Fluor®-647 α-human SSEA4 (1:400) and Tra-1-81 (1:200); Alexa-Fluor®-488 SSEA3 (1:200) and Tra-1-60 (1:200) (BioLegend). Accutase-dissociated single cells were analyzed using a FACSCanto flow cytometer (BD Biosciences) and the FlowJo software program (Tree Star, CA).

RT–PCR and quantitative PCR

RNA was isolated from the CHOPHPS1 iPSCs using the RNeasy micro kit (Qiagen) according to the manufacturer's instructions. Reverse transcription was performed on total RNA (500 ng) using random hexamers and Superscript III Reverse Transcriptase (Life Technologies). RT–qPCR was performed on a LightCycler-480II (Roche, IN). All experiments were performed in triplicate using SYBR-Green qPCR supermix (Roche) according to the manufacturer's instructions. Primers for endogenous genes (DMNT3B, REX1, ABCG2, OCT4, NANOG, and SOX2) were prepared as previously described (Mills et al., 2013). Human genomic DNA, diluted in 10-fold increments from 1 to 100 ng/μl, was used to generate a standard curve to determine PCR efficiency and relative gene expression compared to the housekeeping gene TBP (TATA Binding Box Protein).

In vivo teratoma formation

CHOPHPS1 cells were cultured in feeder-free conditions and dissociated using trypsin. Single cells (~10^6) were resuspended in medium containing growth-factor reduced matrigel (100 μl of 1:3 dilution in IMDM) (Corning), and injected subcutaneously in the neck of NOD/SCID mice. After 6–8 weeks, teratomas were excised and fixed in 4% phosphate-buffered formalin for 24 h. The fixed samples were paraffin embedded and hematoxylin and eosin (H&E) stained for histological analysis. This work was approved by the IACUC committee at CHOP.

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