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Lab resource: Stem cell line

Generation and characterization of human iPSC lines derived from a Primary Hyperoxaluria Type I patient with p.I244T mutation

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ABSTRACT

In this work we describe for the first time the generation and characterization of human induced pluripotent stem cells (hiPSCs) from peripheral blood mononuclear cells (PBMCs) and dermal fibroblasts of a Primary Hyperoxaluria Type I (PH1)-diagnosed patient with p.I244T mutation, which is highly prevalent in Canary Islands due to founder effect. Cell reprogramming was performed using non-integrative Sendai viruses containing the Yamanaka factors and the generated PH1-hiPSC lines (PH1-PBMCs-hiPSC4F1 and PH1-Fib-hiPSC4F1) showed normal karyotypes, silencing of the exogenous reprogramming factors, induction of the typical pluripotency-associated markers and *in vivo* differentiation ability to the three germ layers.

1. Resource table: PH1-PBMCs-hiPSC4F1 and PH1-Fib-hiPSC4F1

Name of stem cell construct:	PH1-PBMCs-hiPSC4F1 PH1-Fib-hiPSC4F1
Institution:	Cell Therapy Program. Center For Applied Medical Research (CIMA). University of Navarra.
Person who created resource:	Juan R. Rodriguez-Madoz
Contact person and email:	jrrodriguez@unav.es
Date archived/stock date:	November 20th, 2014
Origin:	Primary Hyperoxaluria Type I (PH1)-diagnosed patient (p.I244T mutation) peripheral blood mononuclear cells and dermal fibroblasts
Type of resource:	Biological reagent: human induced pluripotent stem cell (hiPSC) line
Sub-type:	Cell line
Key transcription factors:	SOX2, POU5F1, cMYC, KLF4
Authentication:	Identity and purity of cell line confirmed
Link to related literature (direct URL links and full references)	Not available
Information in public databases:	Not available

2. Resource details

We have generated human induced pluripotent stem cell (hiPSC) lines from peripheral blood mononuclear cells (PBMCs) and human dermal fibroblasts of a Primary Hyperoxaluria Type I (PH1)-diagnosed patient with c.731T>C mutation (p.I244T) in AGXT gene, which is highly prevalent in Canary Islands due to founder effect (Santana et al. 2003). PH1-PBMCs-hiPSC4F1 and PH1-Fib-hiPSC4F1 lines were generated using the CytoTune®-iPS 2.0 Reprogramming System (Life Technologies, Invitrogen), which includes the reprogramming factors SOX2, POU5F1, cMYC and KLF4. This reprogramming system is based on a modified and non-transmissible form of Sendai virus (SeV) (Ban et al. 2011). Both PH1-hiPSC lines displayed a typical round shape ESC-like morphology with small and tightly packed cells, with a high nucleus/cytoplasm ratio and prominent nucleoli (Fig. 1A). The presence of the c.731T>C mutation in AGXT gene was confirmed in both PH1-hiPSC lines (Fig. 1B) and the expression of several pluripotency-associated markers was corroborated by qPCR (Fig. 1C), immunofluorescence (Fig. 2A) and FACS analyses (Fig. 2B). Moreover, the absence of exogenous reprogramming transgenes was observed by RT-PCR after 8–10 passages (Fig. 1D). Differentiation capacity into three germ layers was demonstrated by *in vivo* teratoma formation (Fig. 3A). Finally, PH1-hiPSC lines showed normal karyotype (46, XY) (Fig. 3B) and cell line identity was corroborated by DNA fingerprinting.

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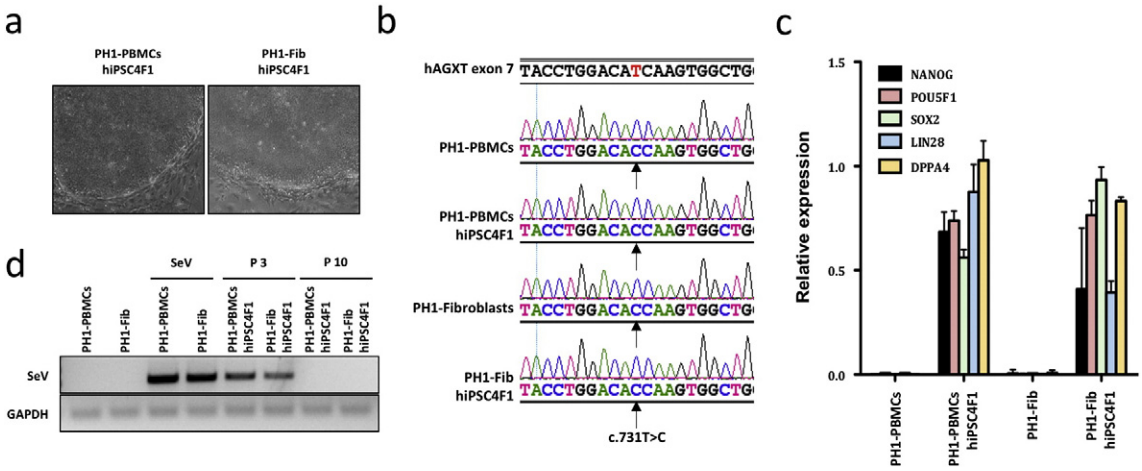

Fig. 1. Characterization of PH1-hiPSC lines. (A) PH1-PBMCs-hiPSC4F1 and PH1-Fib-hiPSC4F1 cells display a typical round shape colony morphology with small, tightly packed cells. (B) Genotyping of the PH1-hiPSC lines. Presence of the c.731T>C mutation in AGXT gene was analyzed sequencing the AGXT exon 7. (C) Endogenous pluripotency-associated markers NANOG, POU5F1, SOX2, LIN28a, and DPPA4 were confirmed by qPCR. Parental PBMCs and fibroblasts were used as negative controls. (D) Silencing of exogenous reprogramming factors was confirmed by RT-PCR.

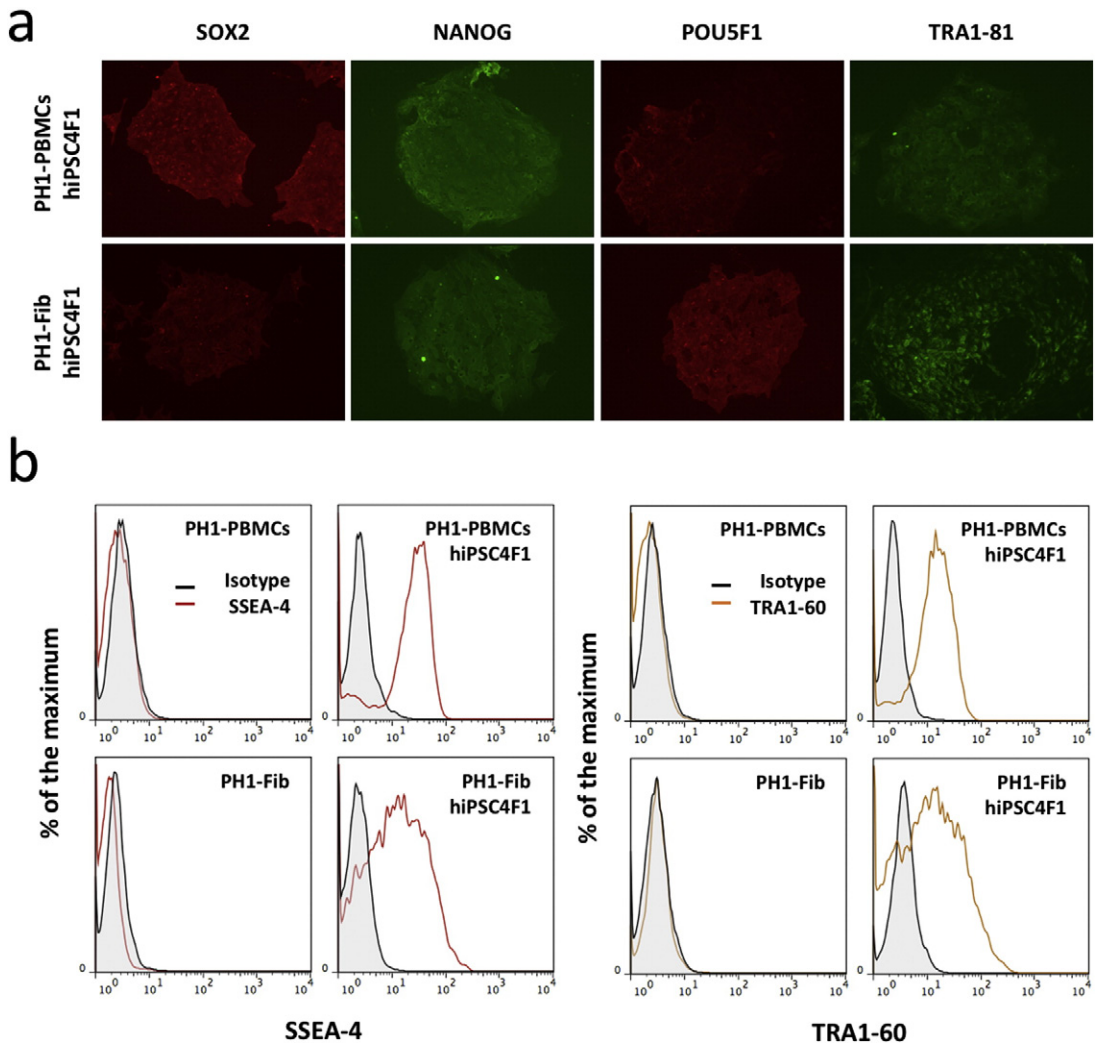

Fig. 2. Expression of pluripotency-associated markers. (A) NANOG, POU5F1, SOX2 and TRA1-81 expression at protein level by immunofluorescence. (B) Expression of TRA1-60 and SSEA4 by FACS analysis. Parental PBMCs and fibroblasts were used as negative controls.