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Lab Resource: Stem Cell Line

Generation of induced pluripotent stem cells (iPSCs) from a Bernard–Soulier syndrome patient carrying a W71R mutation in the *GPIX* gene

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ABSTRACT

We generated an induced pluripotent stem cell (iPSC) line from a Bernard–Soulier Syndrome (BSS) patient carrying the mutation p.Trp71Arg in the *GPIX* locus (BSS1-PBMC-iPS4F4). Peripheral blood mononuclear cells (PBMCs) were reprogrammed using heat sensitive non-integrative Sendai viruses containing the reprogramming factors Oct3/4, SOX2, KLF4 and c-MYC. Successful silencing of the exogenous reprogramming factors was checked by RT-PCR. Characterization of BSS1-PBMC-iPS4F4 included mutation analysis of *GPIX* locus, Short Tandem Repeats (STR) profiling, alkaline phosphatase enzymatic activity, analysis of conventional pluripotency-associated factors at mRNA and protein level and *in vivo* differentiation studies. BSS1-PBMC-iPS4F4 will provide a powerful tool to study BSS.

Resource table.

Name of stem cell line	BSS1-PBMC-iPS4F4
Institution	Gene regulation, Stem Cells and Development Group, GENYO: Centre for Genomics and Oncological Research Pfizer-Universidad de Granada-Junta de Andalucía, PTS Granada
Person who created resource	Lourdes Lopez-Onieva, Pedro J. Real
Contact person and email	Lourdes Lopez-Onieva, lourdes.lopez@genyo.es; Pedro J. Real, pedro.real@genyo.es
Date archived/stock date	September 2015
Origin	Human peripheral blood mononuclear cells
Type of resource	Induced pluripotent stem cell (iPS) line
Sub-type	Cell line
Key transcription factors	Oct3/4, Sox2, cMyc, Klf4
Authentication	Identity and purity of cell line confirmed
Link to related literature	Not available
Information in public databases	Not available
Ethics	Patient informed consent obtained/Ethics Review Board-competent authority approval obtained

Resource details

Bernard Soulier syndrome (BSS) is an inherited autosomal recessive rare platelet disorder caused by mutations in the genes coding for the membrane glycoprotein complex GPIb-IX-V (Berndt and Andrews, 2011). In this study we generated an iPSC line from PBMCs of a BSS patient containing the mutation p.Trp71Arg in the *GPIX* gene (PBMCs-BSS1) (Sánchez-Guiu et al., 2014). This new iPSC line was named BSS1-PBMC-iPS4F4.

We used CytoTune iPS 2.0 Reprograming System (Life Technologies, Invitrogen) that contains the vectors used for delivering and expressing the reprogramming factors Oct3/4, Sox2, Klf4 and c-Myc to reprogram PBMCs-BSS1 (Takahashi et al., 2007; Yu et al., 2007). Sequencing analysis of the *GPIX* locus confirmed the presence of c. 259 T > C change in exon 3 of the *GPIX* gene corresponding to a homozygous p.W71R mutation, identical to PBMCs-BSS1 (Fig. 1A). Additionally, Short Tandem Repeat (STR) profiling confirmed same genetic identity between both samples (Table 1). This iPSC line silenced the expression of exogenous reprogramming transgenes after 8 passages (Fig. 1B) and showed normal karyotype (46, XX) (Fig. 1C). BSS1-PBMC-iPS4F4 colonies displayed typical round shape morphology and they were positive for alkaline phosphatase activity (Fig. 1D).

BSS1-PBMC-iPS4F4 expressed the endogenous pluripotent transcription factors *OCT3/4*, *SOX2*, *REX1* and *NANOG*, assessed by RT-PCR (Fig. 2A) and the protein pluripotent markers SSEA3, SSEA4, Tra1–60,

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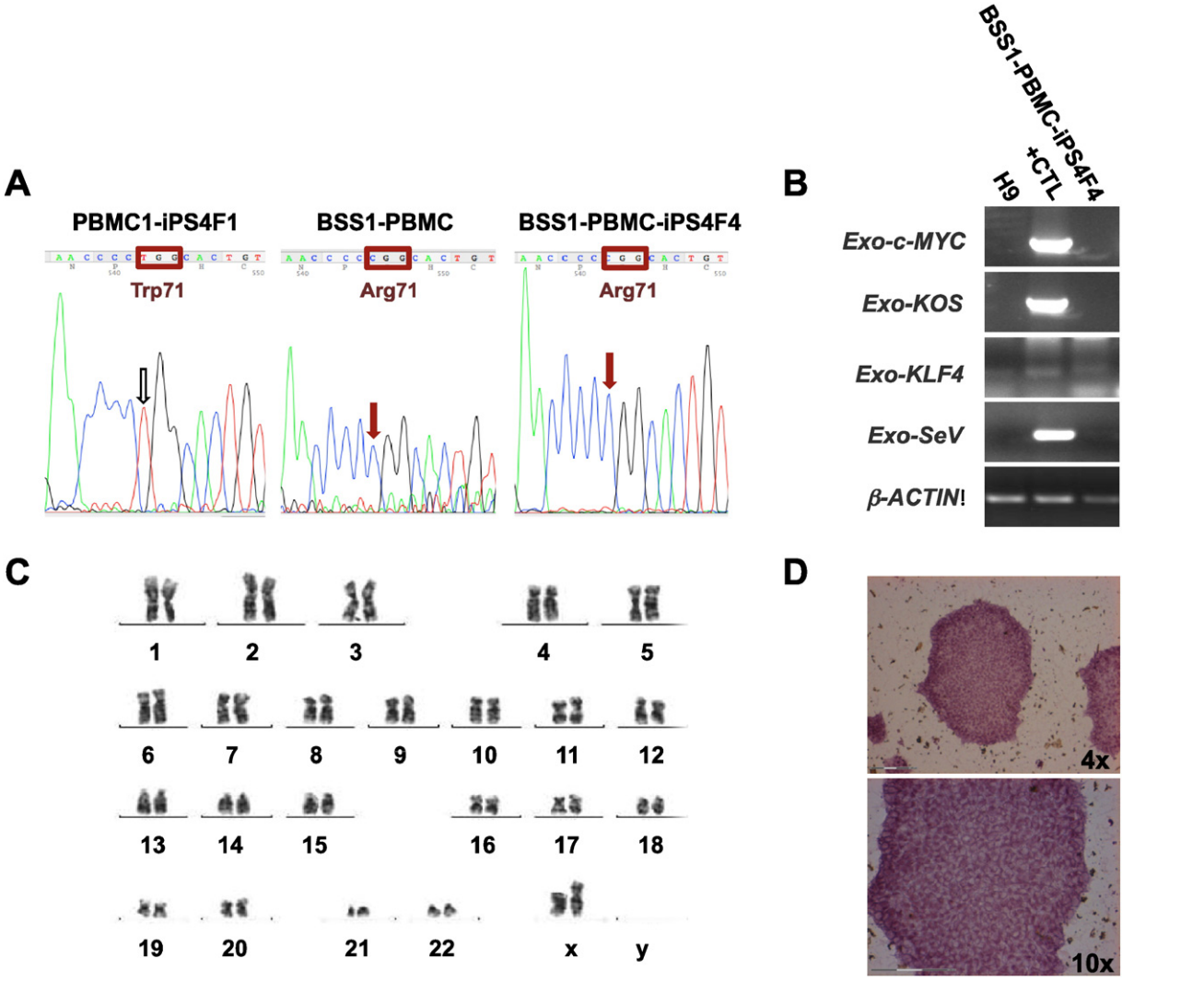

Fig. 1. Characterization of BSS1-PBMC-iPS4F4 cell line. (A) Sequence analysis of c. 259 T > C change in exon 3 of the *GPIX* gene from control iPSC line (PBMC1-iPS4F1) (left panel), BSS1-PBMC (middle panel) and BSS1-PBMC-iPS4F4 (right panel). (B) Silencing of exogenous reprogramming factors and SeV vector confirmed by RT-PCR. H9 cell line was used as a negative control and BSS1-PBMC-iPS4F4 at passage 2 was used as a positive control. (C) GTG-banding shows a normal karyotype in BSS1-PBMC-iPS4F4 cell line. (D) Alkaline phosphatase enzymatic activity staining.

Tra1–81 and Oct3/4, evaluated by flow cytometry analysis (Fig. 2B). To demonstrate the capacity of BSS1-PBMC-iPS4F4 to differentiate into the three germ layers *in vivo*, teratoma formation assays were accomplished (6). As shown in Fig. 2C, teratomas derived from this line showed expression of representative markers of ectoderm (β3-Tubulin), mesoderm (Vimentin) and endoderm (Cytokeratin CK AE1–AE) (Fig. 2C).

Table 1
Short Tandem Repeat (STR) profiling of the original patient cells (BSS1-PBMC) and the iPSC patient-derived cells (BSS1-PBMC-iPS4F4).

Allele	Profile	
	BSS1-PBMC	BSS1-PBMC-iPS4F4
Amelogenin	X	X
CSF1PO	10	10
D3S1358	14, 15	14, 15
D5S818	11, 12	11, 12
D7S820	9, 10	9, 10
D8S1179	11, 16	11, 16
D13S317	8, 11	8, 11
D16S539	1, 12	1, 12
D18S51	13, 14	13, 14
D19S433	13, 14	13, 14
D21S11	30	30
FGA	19, 23	19, 23
TH01	8, 9.3	8, 9.3
TPOX	8, 9	8, 9
vWA	17, 18	17, 18

Materials and methods

Generation of BSS1-PBMC-iPS4 line from PBMCs-BSS1

Peripheral blood sample was obtained from a woman with BSS after informed consent according with the Andalusian Ethics Review Board for Cellular Reprogramming requirements and with Spanish and EU legislation. BSS1-PBMCs were isolated by centrifugation using Ficoll Paque™ PLUS (GE Healthcare). Isolated BSS1-PBMCs were cultured in StemSpan™ SFEM (StemCell Technologies) supplemented with 100 ng/ml hSCF, 100 ng/ml hFLT3L, 20 ng/ml hTPO, 10 ng/ml G-CSF and 2 ng/ml hIL3 (Peprotech) for four days. Then, mononuclear cells were transferred to a 12-well fibronectin coated plate (BD BioCoat™) and Sendai virus (SeV) (CytoTune®-iPS 2.0 Reprogramming kit, Life Technologies, Invitrogen) were added at a multiplicity of infection of three (MOI:3) in the presence of 8 μg/ml Polybrene (Sigma-Aldrich) and 10 μM Y-27,632 (Sigma-Aldrich). Three days after transduction, cells were co-cultured in StemSpan™ SFEM (StemCell Technologies)