Stem Cell Research 16 (2016) 10–13

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab resource: Stem cell line

Transgene-free human induced pluripotent stem cell line (HS5-SV.hiPS) generated from cesarean scar-derived fibroblasts

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ARTICLE INFO

Article history:
Received 26 October 2015
Received in revised form 17 November 2015
Accepted 19 November 2015
Available online 27 November 2015

ABSTRACT

Transgene-free human HS5-SV.hiPS line was generated from human cesarean scar-derived fibroblasts using temperature-sensitive Sendai virus vectors carrying Oct4, Sox2, cMyc and Klf4 exogenous transcriptional factors. The viral constructs were eliminated from HS5-SV.hiPS line through heat treatment. Transgene-free HS5-SV.hiPS cells expressed pluripotent associated transcription factors Oct4, Nanog, Sox2, Rex1 and surface markers SSEA-4, TRA-1-60 and OCT4. HS5-SV.hiPS cells formed embryoid bodies and differentiated into three embryonic germ layers in vivo. HS5-SV.hiPS cells maintained their normal karyotype (46, XX) after culture for extended period. HS5-SV.hiPS displayed the similar pattern of DNA fingerprinting to the parenteral scar-derived fibroblasts.

Resource Table: HS5-SV.hiPS cell line.

Name of stem cell construct	HS5-SV.hiPS
Institution	Chulalongkorn University
Person who created resource	Ruttachuk Rungsiwiwut, Kamthorn Pruksananonda
Contact person and email	Ruttachuk Rungsiwiwut, ruttachuk.r@chula.ac.th Kamthorn Pruksananonda, pkamthorn@yahoo.com
Date archived/stock date	March 15, 2015
Origin	Human cesarean scar-derived cells
Type of resource	Biological reagent: induced pluripotent stem cell (iPS) line
Sub-type	Cell line
Key transcription factors	Oct4, Sox2, cMyc, Klf4
Authentication	Identity and purity of cell line confirmed
Link to related literature (direct URL links and full references)	N/A
Information in public databases	N/A

Resource details

Human cesarean scar-derived fibroblasts (HCSFs) were isolated from the cesarean scar tissue (Fig. 1A), donated from a female patient undergoing her second cesarean operation, and with informed consent. To generated hiPSCs, HCSFs were infected with temperature-sensitive strain (TS7) Sendai virus (SeV) vectors, carrying exogenous transcriptional factors including Oct4, Sox2, cMyc and Kfl4 (Ban et al. 2011; Fusaki et al. 2009). Approximately 10 days after reprogramming, primary hiPS-like colonies were observed (Fig. 1B). At passage number 5, hiPSCs were cultured in 38.5 °C, CO_2 incubator for 7 days in order to eliminate the SeV constructs. The absence of Sendai virus was confirmed by using immunocytochemistry (Fig. 1C). Undifferentiated HS5-SV.hiPS line (Fig. 1D) was routinely co-cultured with human foreskin fibroblast feeders and propagated by using mechanical splitting.

Transgene-free HS5-SV.hiPS expressed surface markers (2A) and pluripotent associated transcription factors Oct4, Nanog, Sox2, Rex1 (Fig. 2B). HS5-SV.hiPS formed embryoid bodies (Fig. 2C) and teratoma tissue consisted of three embryonic germ layers (Fig. 2D). HS5-SV.hiPS maintained their normal karyotype of 46, XX (Fig. 3A) after culturing for an extended period. HS5-SV.hiPS displayed the similar pattern of DNA fingerprinting to the parenteral scar-derived fibroblasts (Fig. 3B).

1. Materials and methods

1.1. Generation of transgene-free HS5-SV.hiPS line from human cesarean-scar derived fibroblasts

The experimental protocols of the present study were approved by the Institutional Ethical Committee of Faculty of Medicine, Chulalongkorn University (IRB No 301/55). The human cesarean-scar tissue was donated by a female patient undergoing her second cesarean

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http://dx.doi.org/10.1016/j.scr.2015.11.006

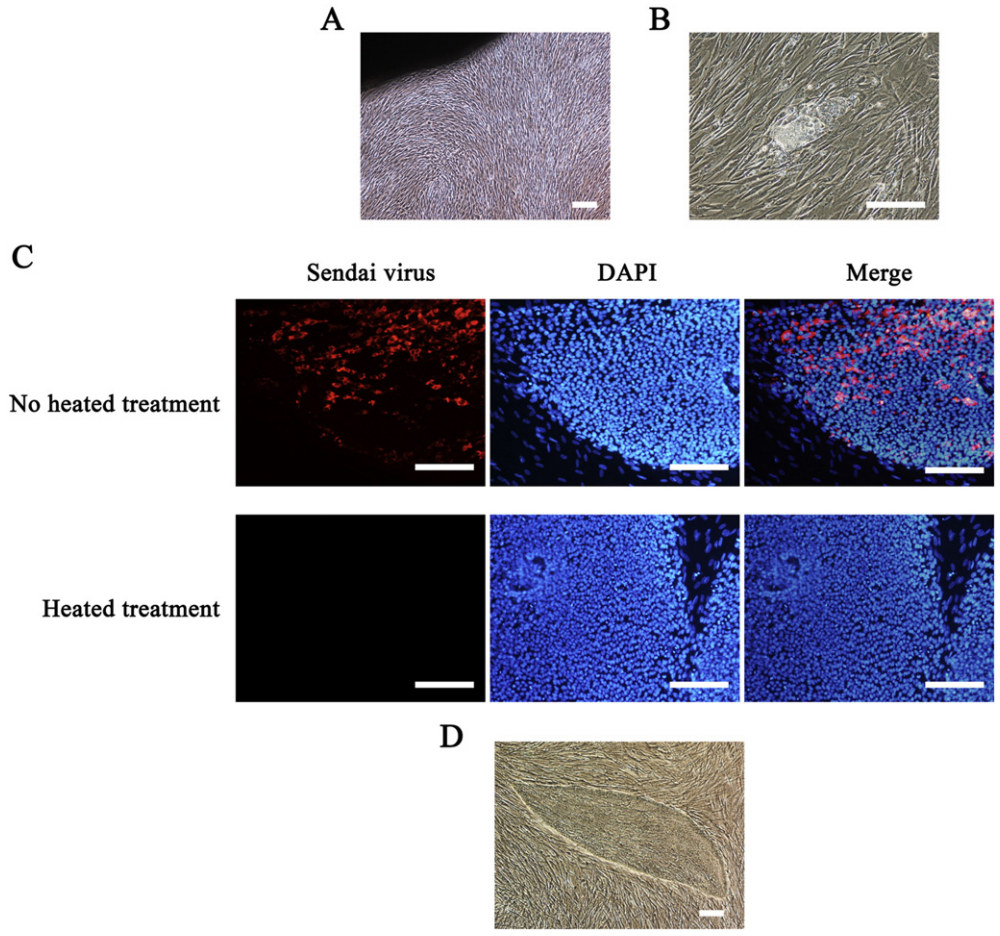

Fig. 1. Generation of HS5-SV.hiPS cell line from human cesarean-scar derived fibroblasts. (A)Fibroblast cells isolated from cesarean-scar tissue. (B) Approximately 10 days after reprogramming, primary hiPS-like colonies were observed. (C) Immunofluorescence staining of Sendai virus in heat-treated HS5-SV.hiPS and no heat-treated HS5-SV.hiPS. (D) HS5-SV.hiPS cell line displays typical colony morphology with defined colony boundary and tightly packed cells. Scale bar = 200 μm.

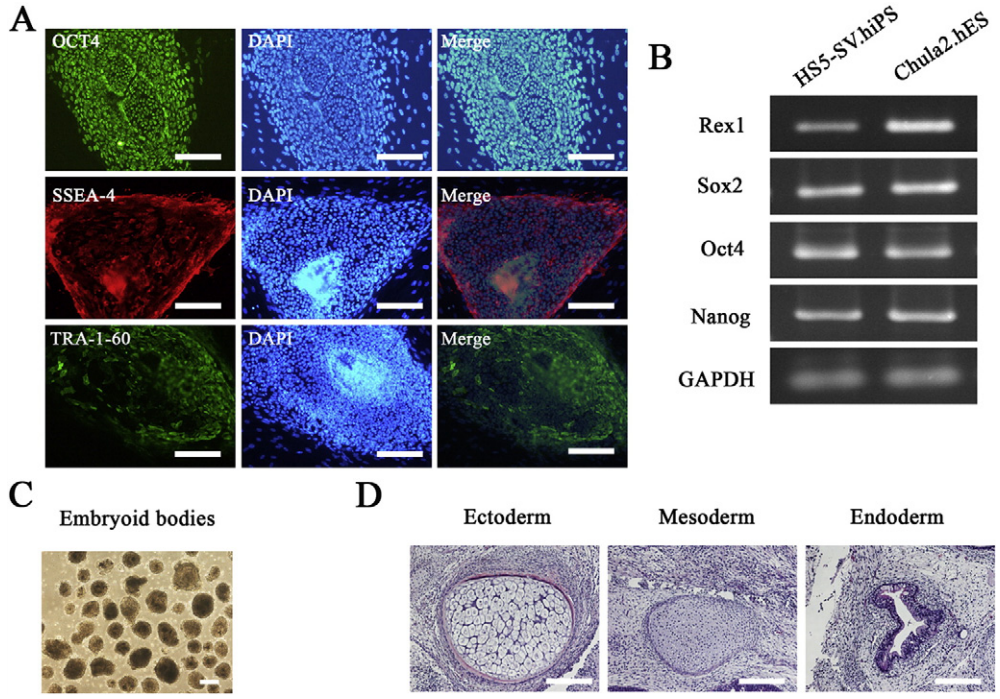

Fig. 2. Characterization of HS5-SV.hiPS cell line. (A) HS5-SV.hiPS expressed pluripotent associated transcriptional factors Oct4, Nanog, Sox2 and Rex1. (B) HS5-SV.hiPS was immunostained for pluripotent markers SSEA-4, TRA-1-60 and OCT4. (C) HS5-SV.hiPS formed embryoid bodies. (D) HS5-SV.hiPS differentiated to ectodermal-, mesodermal- and endodermal structures resembling of embryonic three germ layers in teratoma tissue. Scale bar = 200 μm.