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Lab Resource: Stem Cell Line

Generation of induced pluripotent stem cells (iPSCs) from a retinoblastoma patient carrying a c.2663G>A mutation in RB1 gene

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ABSTRACT

Skin fibroblasts were obtained from a male patient diagnosed with retinoblastoma (RB) carrying a c.2663G>A mutation in the 25 exon of RB1 gene. RB-iPS cells was generated via delivered four reprogramming factors (OCT4, SOX2, NANOG and LIN28) into these skin fibroblasts. The RB-iPS cells retained the RB1 heterozygous mutation resulted in a truncated RB1 mRNA. Characteristic tests proved that the iPSC line presented typical markers of pluripotency and had the capability to form the three germ layers in vitro.

Resource table

Name of stem cell construct	RB-iPS cell line
Institution	National Engineering and Research Center of Human Stem Cell
Person who created resource	Yan Zhao
Contact person and email	Sicong Zeng: sicong520@csu.edu.cn
Date archived/stock date	Mar 20, 2016
Origin	Human skin fibroblasts
Type of resource	Biological reagent: induced pluripotent stem cell (iPS); derived from RB1 p.S888A heterozygous mutation male patient
Sub-type	Induced pluripotent stem cells (iPSCs)
Key transcription factors	OCT4, SOX2, NANOG, LIN28
Authentication	Identity and purity of cell line confirmed (Fig. 1)
Link to related literature (direct URL links and full references)	N/A
Information in public databases	N/A

Resource details

Induced pluripotent stem cells (iPSCs) was derived from a male retinoblastoma patient after informed consent. Sequencing analysis confirmed a heterozygous missense mutation c.2663G>A (p.S888A) in the 25 exon of RB1 in RB-iPS cells (Fig. 1A). This mutation located within exon and intron junction which may cause the alternative splicing. By using primers that spans the junction of exons 23 and 26, two different size products of RB1 gene were found in RB patient-derived skin fibroblast cells (RB-SF) and RB-iPS cells, but not in the normal skin fibroblast cells (N-SF) (Fig. 1B). Similar to the human embryonic stem cell line (chHES-8) which were obtained from the established hESC bank at our center (Lin et al., 2009), the RB-iPS cells showed high telomerase activity compare to the skin fibroblasts and expressed the pluripotency related genes by RT-PCR (Fig. 1C–D). During long-term culture on the mitotically inactivated mouse embryonic fibroblasts (MEFs), the RB-iPS cells maintained a stable karyotype 46, XY (Fig. 1E), and were positive for OCT4, NANOG, SSEA4, TRA-1-60 and TRA-1-81 as well as alkaline phosphatase (Fig. 1F). The differentiation capacity of RB-iPS cells was confirmed through in vitro assays. The cells from embryoid bodies expressed the key genes related with the development of main organs from all three germ layers, such as ectoderm marker β-TUBULIN, mesoderm marker SMA and endoderm marker AFP (Fig. 1G).

Materials and methods

Ethical approval

All procedures described in this work were approved by the ethical committee of Reproductive and Genetic Hospital of CITIC-Xiangya. The skin biopsy used for generation of the induced pluripotent stem cell line were isolated from a RB-diagnosed patient with c.2663G>A mutation in RB1 gene (p.S888A) after written informed consent.

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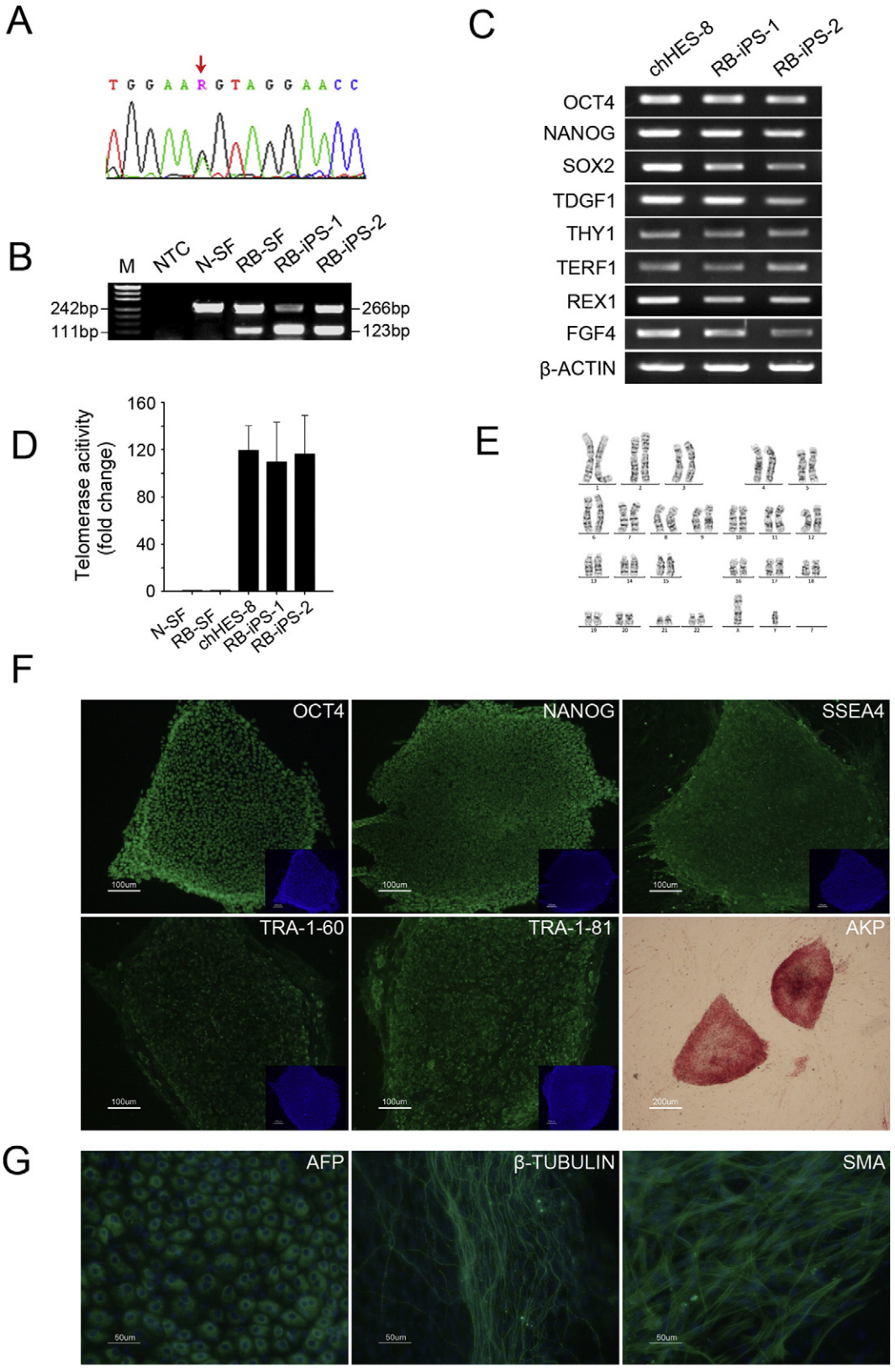

Fig. 1. Characterization of RB-iPS cell line. (A) Sequencing of exon 25 of the RB1 gene in RB-iPS cells showing a heterozygous c.2663G>A substitution (red arrow). (B) Heterozygous deletion of RB1 assessed by RT-PCR. The RB-iPS-1 and RB-iPS-2 cells exhibited two bands of 266 bp and 123 bp, resemble the skin fibroblast from RB patient (RB-SF), indicating the heterozygous mRNA deletion between the exon 23 and the exon 26 of RB1 gene. The normal human skin fibroblast (N-SF) exhibited only one band of 266 bp, indicating no heterozygous mRNA deletion; NTC, non-template control; M, DNA Marker. (C) Gene expression analysis by Semi-quantitative RT-PCR. Pluripotent genes were detected in the undifferentiated RB-iPS cells. (D) Quantitative analysis of telomerase activity in RB-iPS cells, hESCs and SF. (E) RB-iPS cells had a normal karyotype 46, XY. (F) RB-iPS colonies were positive for OCT4, NANOG, SSEA4, TRA-1-60, TRA-1-81 and AKP. Scale bar = 100 μm. (G) Derivatives from embryoid bodies of RB-iPS cells could differentiate into ectoderm (β-TUBULIN), mesoderm (SMA) and endoderm (AFP) in vitro. Scale bar = 50 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)