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Lab Resource: Stem Cell Line

Generation of a human induced pluripotent stem cell line CERAi001-A-6 using episomal vectors

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ABSTRACT

We report the generation of the hiPSC line CERAi001-A-6 from primary human dermal fibroblasts. Reprogramming was performed using episomal vector delivery of OCT4, SOX2, KLF4, L-MYC, LIN28 and shRNA for p53.

Resource table

Name of Stem Cell line	CERAi001-A-6
Institution	Centre for Eye Research Australia, University of Melbourne
Person who created resource	Dr. Raymond Wong
Contact person and email	Assoc. Prof. Alice Pébay (apebay@unimelb.edu.au)
Date archived/stock date	20th August, 2014
Origin	Human skin biopsy-derived fibroblasts
Type of resource	Biological reagent: human induced pluripotent stem cells (iPSCs) derived using skin fibroblasts from healthy subject
Sub-type	hiPSC line
Key transcription factors	OCT4, SOX2, KLF4, L-MYC, LIN28, shRNA for p53
Authentication	Identity and purity of cell line confirmed (Fig. 1)
Link to related literature	n/a
Information in public databases	n/a
Ethics	Patient informed consent obtained/Ethics Review Board-competent authority approval obtained

Resource details

Epidermal fibroblasts were obtained from skin biopsies of a 50 years old healthy male subject (EPS1133). Reprogramming was performed on patient fibroblasts using non-integrating episomal vectors to deliver OCT4, SOX2, KLF4, L-MYC, LIN28 and shRNA to p53 (Piao et al., 2014; Wang et al., 2016). A clonal hiPSC line (CERAi001-A-6 hiPSC) was established and further characterized for pluripotency.

Characterization of CERAi001-A-6 hiPSCs demonstrated that the cells were positive for the pluripotent markers TRA-1-60 and OCT4 (Fig. 1A and B, respectively). Upon embryoid body formation, CERAi001-A-6 hiPSCs differentiated into cells representative of ectoderm (NESTIN positive cells, Fig. 1C), mesoderm (SMA positive cells, Fig. 1D) and endoderm (AFP positive cells, Fig. 1E). RT-PCR analysis demonstrated gene expression of *NANOG* and endogenous *OCT4* and *SOX2*, suggesting successful reprogramming of CERAi001-A-6 hiPSCs (Fig. 1F). Altogether, these results demonstrate that the CERAi001-A-6 hiPSCs retain the potential to differentiate *in vitro* into the three germ layers. To ensure the genetic integrity of the CERAi001-A-6 hiPSCs generated, we performed genome-wide copy number variation (CNV) profiling using Illumina HumanCore Beadchip. Our results demonstrate that the parental fibroblasts and CERAi001-A-6 hiPSCs were both male (XY) and exhibited a normal diploid chromosomal content (Fig. 1G). Furthermore, microsatellite analysis confirmed that the CERAi001-A-6 hiPSCs originated from the parental patient fibroblasts (Fig. 1H). The established CERAi001-A-6 is negative for mycoplasma testing (Fig. 1I).

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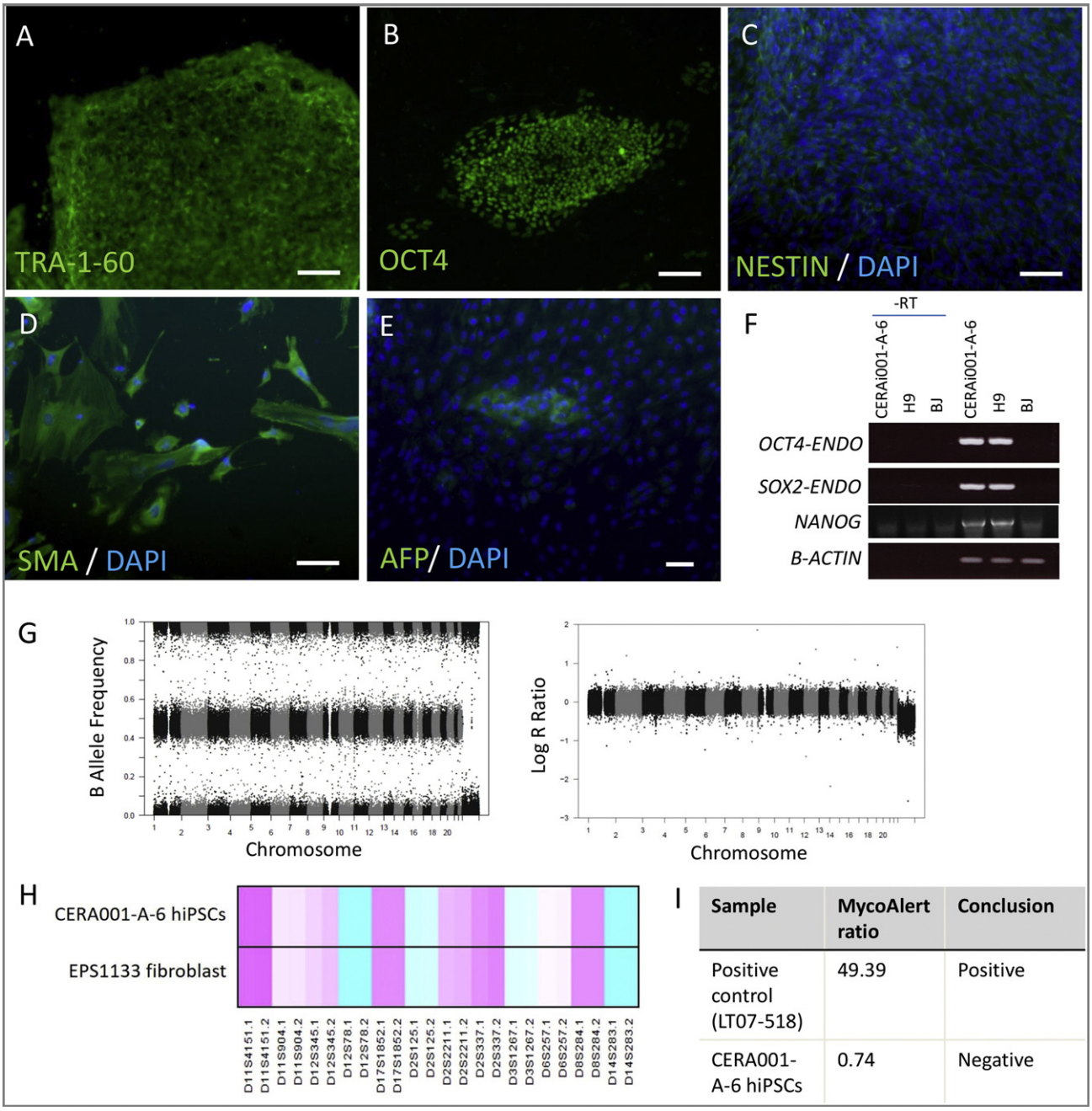

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Sample	MycoAlert ratio	Conclusion
Positive control (LT07-518)	49.39	Positive
CERA001-A-6 hiPSCs	0.74	Negative

Fig. 1. Characterization of CERAi001-A-6 hiPSCs. Immunocytochemistry of A) TRA-1-60 and B) OCT4 in CERAi001-A-6 hiPSCs. Following embryoid body differentiation, cells representative of the three germ layers were detected, including C) ectodermal (NESTIN-positive), D) mesodermal (SMA-positive) and E) endodermal (AFP-positive) cells. (C–E): DAPI counterstained (blue). Scale bars = 100 μm. F) RT-PCR showed expression of endogenous *OCT4*, *SOX2* and *NANOG* in CERAi001-A-6 hiPSCs and H9 human embryonic stem cells, but absent in BJ fibroblasts and control without reverse transcriptase (-RT). G) CNV analysis confirmed a normal diploid karyotype in CERAi001-A-6 hiPSCs. H) Heatmap of microsatellite analysis using 12 polymorphic markers confirmed that CERAi001-A-6 hiPSCs originated from the parental patient fibroblasts. I) CERAi001-A-6 hiPSCs is negative for mycoplasma contamination. MycoAlert ratio of >1.2 is considered positive for mycoplasma.

Materials and methods

Ethics

Collection of patient samples and generation of hiPSCs were approved by the Human Research Ethics committees of the Royal Victorian Eye and Ear Hospital (11/1031H, 13/1151H-004) and carried out in accordance with the requirements of the National Health & Medical Research Council of Australia and conformed with the Declarations of Helsinki (McCaughey et al., 2016).

Cell culture

Patient fibroblasts were cultured in DMEM medium supplemented with 10% fetal calf serum, 1 × L-glutamine, 0.1 mM non-essential amino acids and 0.5× penicillin/streptomycin (all from Invitrogen). CERAi001-A-6 hiPSCs were cultured on mitotically inactivated mouse embryonic fibroblasts feeders in the presence of DMEM/F-12 medium containing 1 x GlutaMAX, 20% knockout serum replacement, 10 ng/ml basic fibroblast growth factor, 0.1 mM nonessential amino acids, 100 μM β-mercaptoethanol and 0.5× penicillin/streptomycin (all from Invitrogen).

hiPSC generation and characterization

Skin fibroblasts were reprogrammed to generate hiPSCs as previously described (Piao et al., 2014; Hung et al., 2016). Briefly, 600,000 skin fibroblasts was nucleofected with episomal vectors (pCXLEhOCT3/4-shp53, pCXLE-hSK, pCXLE-hUL, all from Addgene), using the Nucleofection kit for primary fibroblasts with program T-016 (Lonza). The nucleofected cells were plated down onto gelatinized plate in fibroblast medium. On day 7, 100,000 fibroblasts were replated onto a 100 mm dish with MEF with hiPSC medium. On day 34 post-reprogramming, we isolated hiPSC colonies by manual dissection. The established CERAi001-A-6 hiPSCs were further expanded and characterized for pluripotency (Wong et al., 2011). RT-PCR is performed to analyse *NANOG*, endogenous *OCT4* and *SOX2* expression using the following primers: OCT4-ENDO: GACAGGGGGAGGGGAGGAGCTAGG, CTTCCCTCCAACCAGTTGCCCCAAAC; SOX2-ENDO: GGGAAATGGGAG GGGTGCAAAAGAGG, TTGCGTGAGTGTGGATGGGATTGGTG; NANOG: CAGCCCCGATTCTTCCACCAGTCCC, CGGAAGATTCCCAGTCGGGTTCACC; β-ACTIN: CCCTGGCACCCAGCAC, GCCGATCCACACGGAGTAC. CNV analysis was performed using the Illumina HumanCore Beadchip SNP array. Both the parental fibroblasts and CERAi001-A-6 hiPSCs were used for CNV analysis.

For *in vitro* differentiation, CERAi001-A-6 hiPSCs were allowed to form embryoid bodies in suspension for 12 days, followed by attachment on gelatinized dishes for further differentiation for 15 days. Differentiation markers (SMA, NESTIN and AFP) were analysis to detect cell representative of the three germ layers.

Immunocytochemistry

Immunocytochemistry analysis was performed using standard procedures. Samples were immunostained with primary antibodies TRA-1-60 (5 μg/ml, MAB4360, Millipore), OCT4 (5 μg/ml, sc-5279, Santa Cruz Biotechnology), SMA (10 μg/ml, MAB1420, R&D Systems), NESTIN (10 μg/ml, AB22035, Abcam) or AFP (10 μg/ml, ST1673, Millipore). Subsequently, the samples were immunostained by the appropriate Alexa Fluor-488 antibodies and DAPI nuclear counterstain. Fluorescent images were taken using a Nikon Eclipse TE2000 inverted microscope.

Microsatellite analysis

Microsatellite analysis was performed using the Applied Biosystems Linkage Mapping Set (Australian Genomics Research Facility). Twelve polymorphic markers were assessed, including D2S2211, D2S125, D2S337, D3S1267, D6S257, D8S284, D11S904, D11S4151, D12S78, D12S345, D14S283 and D17S1852. The results were presented as a heatmap using R.

Mycoplasma testing

Mycoplasma test is performed using the MycoAlert kit (Lonza) following the manufacturer's instruction.

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