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Lab resource: Stem cell line

Generation of transgenic human embryonic stem cell line BJNhem20–OCIAD1-OV

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ABSTRACT

Ovarian Carcinoma Immuno-reactive Antigen domain containing protein 1 (OCIAD1) was overexpressed in BJNhem20 human embryonic stem cell line (hESC) using plasmid transfection, followed by stable cell line generation. The construct pCAG-OCIAD1 was introduced into hESCs by microporation.

1. Resource table

Name of stem cell lines	BJNhem20–OCIAD1-OV
Institution	Jawaharlal Nehru Centre for Advanced Scientific Research
Person who created resource	Deeti Shetty and Maneesha S. Inamdar
Contact person and email	inamdar@jncasr.ac.in
Date archived/stock date	15th June, 2015
Origin	Human embryonic stem cell line BJNhem20
Type of resource	Biological reagent: genetically modified human embryonic stem cells
Sub-type	Cell line
Key transcription factors	Oct4, Nanog, Sox2
Authentication	Identity of the cell line confirmed (see Fig. 1)
Link to related literature	http://online.liebertpub.com/doi/abs/10.1089/scd.2008.0131 http://link.springer.com/article/10.1007%2Fs11626-010-9277-3 http://link.springer.com/protocol/10.1007%2F978-1-61779-794-1_9
Information in public databases	–
Ethics	Competent authority approval obtained

2. Resource details

BJNhem20–OCIAD1-OV line was generated by transfection of BJNhem20 human embryonic stem cells (hESCs) with pCAG-OCIAD1 construct using microporation technique with the conditions of 1100 V, 20 ms pulse width and 2 pulses. The pCAG-OCIAD1 construct was generated by cloning OCIAD1 Open Reading Frame amplicon from amino acids 1–245 between Xho1 and Not1 sites using appropriate primers. A stable hESC line was generated after subjecting these transfected cells to puromycin selection for two weeks.

OCIAD1 overexpression was validated by immunostaining and Western blotting in three biological replicates.

Expression of pluripotent stem cell markers OCT4, SSEA3/4 and TRA1-81 has been shown by immunostaining. Differentiation of these hESC lines to all the three germ layers was demonstrated by immunostaining for different markers such as AFP (endoderm), Brachyury (mesoderm) and β-III tubulin (ectoderm). Karyotype was also checked in these cells and found to be normal.

3. Materials and methods

3.1. Cell culture

hESC line BJNhem20 was derived from discarded Grade III embryos and tested for pluripotency (Inamdar et al., 2009). This cell line has been cultured continuously for over 200 passages without acquiring any karyotypic abnormality (Venu et al., 2010). The cell line was included in the analysis of genetic stability in the International Stem Cell Initiative project (ISCI2, 2011). Cultures of BJNhem20 and the transgenic line BJNhem20–OCIAD1-OV were maintained on mouse embryonic fibroblast feeder layers in hESC medium composed of KnockOut Dulbecco's modified Eagle medium (KO-DMEM—Life Technologies Cat no. 10829-018) supplemented with 20% KnockOut Serum replacement (KOSR—Life Technologies Cat no. 10828-028), 1% GlutaMAX (Life Technologies Cat no. 35050-061), 1% non-essential amino acids (MEM-NEAA-Life Technologies Cat no. 11140-050), 0.1% β-mercaptoethanol (Life Technologies Cat no. 21985-023) and 8 ng/ml human recombinant basic fibroblast growth factor (human bFGF—Sigma Aldrich Pvt. Ltd. Cat no. F-0291).These cells were routinely passaged by mechanical cutting of undifferentiated colonies.

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1873-5061/

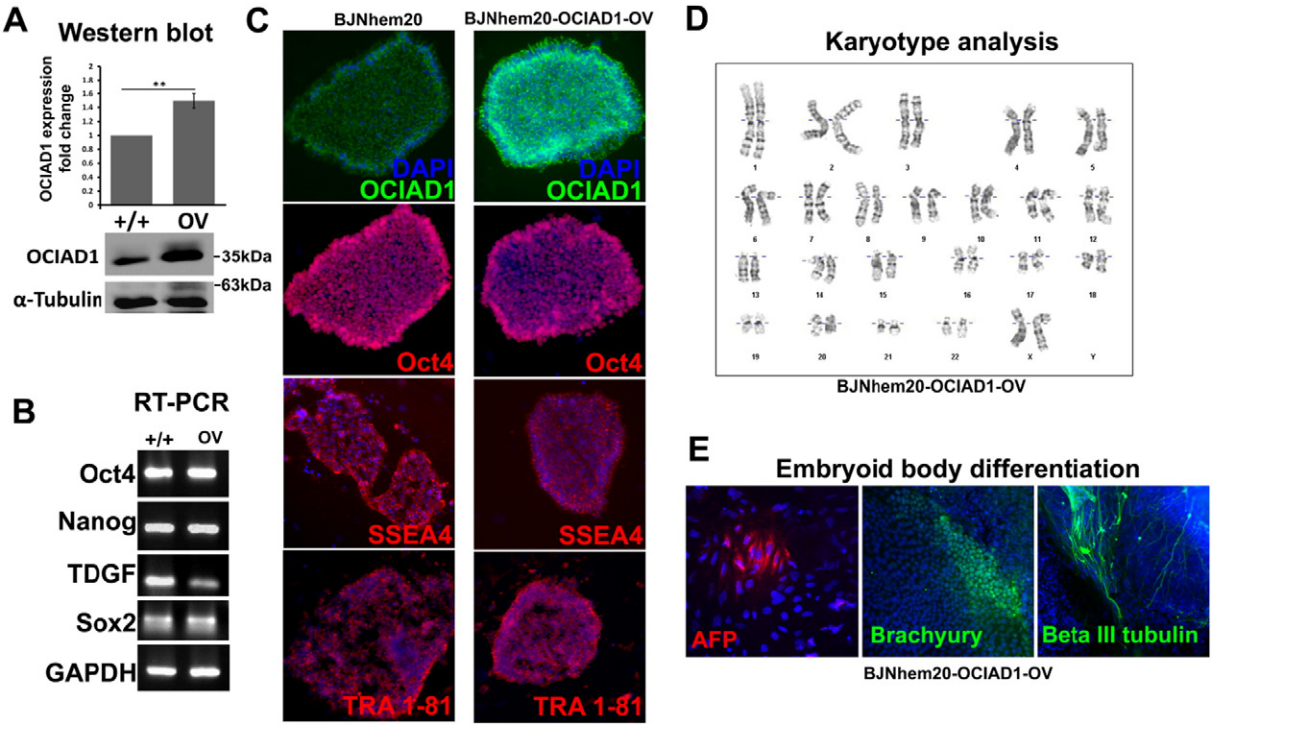

Fig. 1. Expression and karyotype analysis of BJNhem20–OCIAD1-OV hESC line: (A) Western blot analysis showing overexpression of OCIAD1 in the OV hESC line as compared to wild type. Graph represents standard error mean after analysis of three biological replicates using single factor ANOVA (P value 0.003). (B) Analysis of transcript levels of pluripotency marker genes *Oct4*, *Nanog*, *TDGF* and *Sox2* by reverse transcription and polymerase chain reaction (RT-PCR) amplification. *GAPDH* was used to normalize transcript levels. (C) Immunostaining analysis of OCIAD1-OV for overexpression of OCIAD1 and for pluripotency markers Oct4, SSEA4 and TRA1-81 as indicated, compared to parental line BJNhem20 hESC line. (D) Karyotypic normality validation of BJNhem20–OCIAD1-OV. (E) Differentiation analysis of BJNhem20–OCIAD1-OV: Embryoid bodies stained to show differentiation to all the three germ layers by immunostaining for AFP, Brachyury and βIII-Tubulin marking the endoderm, mesoderm and ectoderm respectively.

3.2. Microporation of hESCs to generate BJNhem20-OCIAD1-OV cell line

For microporation, BJNhem20 cells were grown on Matrigel (Becton Dickinson Catalogue No. 354277) coated dishes till they were 60–70% confluent. The cells were trypsinized with 1X TrypLE (Life Technologies Cat No. 12605-010) at 37 °C for 4 min. The cells were pelleted by centrifugation at 1000 rpm for 2 min and washed with phosphate buffered saline and enumerated. 0.5 million cells were resuspended in 10 μl of R buffer and 6 μg of DNA was mixed and the cells were transfected in a microporator (Neon Microporation Kit — Life Technologies Cat no. MPK1096). Microporation conditions used were 1100 V, 20 ms and 2 pulses according to the manufacturer's instructions. The cells were then directly seeded onto Matrigel-coated dishes with mTESR media (Stem Cell Technologies Cat no. 05850) supplemented with ROCK inhibitor (10 μM Y-27632, Sigma Aldrich Pvt. Ltd. Cat no. Y0503) to aid the survival of single cells. mTESR medium without ROCK inhibitor was used after 12–16 h when the cells adhered. 48 h after transfection, puromycin (0.5 μg/ml Sigma Aldrich Pvt. Ltd. P8833) was added to the transfected culture and selection was continued for 10 days. Puromycin resistant individual clones of transgenic OCIAD1 overexpressing hESCs were manually picked and passaged. BJNhem20–OCIAD1-OV cells were subsequently cultured in both feeder free conditions on Matrigel in the presence of mTESR medium and on feeders with regular hESC medium.

3.3. Embryoid body differentiation

Differentiation of embryoid bodies was carried out as described before (Inamdar et al., 2009). Briefly, colonies grown on MEFs were mechanically cut and pieces cultured in human embryoid body media (10% FBS- Hyclone-SH30070 with β-mercaptoethanol and GlutaMAX in DMEM) on low attachment plates. These hESC aggregates were allowed to grow in suspension for 3 days after which the colonies formed embryoid body-like structures and were transferred to the tissue culture dishes pre-treated with 0.1% gelatin and allowed to attach and differentiate spontaneously till the time for harvest from day 6 onwards for immunostaining analyses.

3.4. Immunostaining

hESC and hEBs were fixed in 2% paraformaldehyde (in PBS), permeabilized with Triton-X 100 (0.1% for cells and 0.3% for EBs) and then blocked with 4% FBS for 1 h at RT (Room Temperature). Post blocking, cells were incubated with the appropriate primary antibodies overnight at 4 °C, then were washed with PBS at RT and incubated with secondary antibody conjugated to either Alexa Fluor 488 or 568 for 1 h at RT (Dilution 1:400 Invitrogen). The cells were washed and stained using DAPI (1:500 in M1 buffer Invitrogen) to visualize nuclei. The images were acquired using an epifluorescence microscope (IX-81 microscope system (Olympus)) fitted with a cooled CCD camera (CoolSnap, Roper Scientific). Primary antibodies used were against OCIAD1 (Abcam Ab91574), Oct4 (BD Biosciences BD 611203), TRA1-81 and SSEA4 monoclonal antibodies were a kind gift from Peter Andrews (University of Sheffield, UK), Brachyury (Santa Cruz Biotech Cat no. SC-17743), β-III tubulin, AFP (Sigma Chemical Pvt. Ltd. Cat no. A8452).

3.5. RNA isolation/cDNA synthesis and PCR

Total RNA was extracted from cells by TRIzol reagent (Life Technologies Cat no. 15596-026) following the manufacturer's instructions. 2 μg of DNase treated RNA was converted to cDNA by performing reverse transcription using Superscript II (Life Technologies Cat no. 18064-014). Primer sequences used for cDNA amplification (5′-3′) were the following:

Primer name	Sequence
OCT4_F	GAAGGTATTCAGCCAAACGAC
OCT4_R	GTTACAGAACCACACTCGGA
NANOG_F	TGCAAATGTCTTCTGCTGAGAT
NANOG_R	GTTCAGGATGTTGGAGAGTTC
GAPDH_F	GTCCATGCCATCACTGCCA
GAPDH_R	TTACTCCTTGGAGCCATG

4. Verification of karyotype

Standard G banding of metaphase spreads was performed and analyzed using CytoVision software. For each sample, at least 50 metaphases were counted and 10 were analyzed.

Acknowledgments

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References

Inamdar, M., Venu, P., Srinivas, M., Rao, K., Vijayraghavan, K., 2009. Derivation and characterization of two sibling human embryonic stem cell lines from discarded grade III embryos. Stem Cells Dev. (http://online.liebertpub.com/doi/abs/10.1089/scd.2008.0131).

Venu, P., Chakraborty, S., Inamdar, M., 2010. Analysis of long-term culture properties and pluripotent character of two sibling human embryonic stem cell lines derived from discarded embryos. In Vitro Cell. Dev. Biol. Anim. (http://link.springer.com/article/10.1007%2Fs11626-010-9277-3).

ISCI2, Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage. The International Stem Cell InitiativeNature Biotechnology 2.