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Lab Resource: Stem Cell Line

Generation of induced pluripotent stem cells from Chinese hamster embryonic fibroblasts

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ABSTRACT

We derived a stable cell line from Chinese hamster embryonic fibroblasts by transduction of four mouse transcription factors (M_3O, *Sox2*, *Klf4*, and *n-Myc*) using a lentiviral vector. The cell line possess all the characteristics of an induced pluripotent stem cell (iPSC) line. Given that Chinese hamster ovary (CHO) cells are the predominant host cells used for therapeutic protein production and no pluripotent stem cell line or other normal cell line has been isolated from Chinese hamster, this iPSC line may serve as a useful tool for research using CHO cells or even be used for deriving new cell lines.

Resource table

Name of stem cell line	CHiPSC-B
Institution	University of Minnesota
Contact information of distributor	Wei-Shou Hu, acre@umn.edu
Type of cell line	Inducible pluripoten stem (iPS) cell
Origin	Chinese hamster (*Cricetulus griseus*)
Cell Source	Chinese hamster embryonic fibroblasts
Method of reprogramming	Lentivirus
Name of transgene or resistance	M_3O, Sox2, Klf4, n-Myc
Inducible/constitutive system	CMV promoter, pLOVE system
Date archived/stock date	2014–2015

Resource utility

The work reported here can be applied to producing genetically modified Chinese hamster or offer a template for establishing Chinese hamster ESCs. The cell line can serve as the normal diploid cell reference for exploring genome engineering of CHO cells, and has an immediate impact on biomanufacturing of therapeutic proteins.

Resource details

Recombinant cell lines derived from CHO cells are used for the production of the majority of therapeutic proteins, including monoclonal antibodies, erythropoietin, Factor VIII. In spite of their industrial and scientific importance, no Chinese hamster induced pluripotent stem cells (CHiPSCs) have been established. Since no Chinese hamster embryonic stem cell (ESC) line has been isolated, the culture conditions for maintaining pluripotent state are not known.

Chinese hamster embryonic fibroblasts (CHEFs) (Fig. 1A) were transduced with the lentiviral vector cocktail of the mouse transcription factors M3O (Hirai et al., 2011), Sox2, Klf4, and n-Myc (*SKM*). Colonies with an ESC-like morphology first became visible around 6 days after transduction. Between 100 and 200 colonies emerged eventually from 10^5 cells plated in a well. The isolated ESC-like cells (CHiPSCs-B) were similar to mouse ESCs in morphology at the individual cell as well as the colony level. They had smooth, refractile defined colony borders and a compact internal architecture comprised of small, tightly packed round cells (Fig. 1B). CHiPSCs-B was positive for *Nanog* promoter-GFP reporter assay

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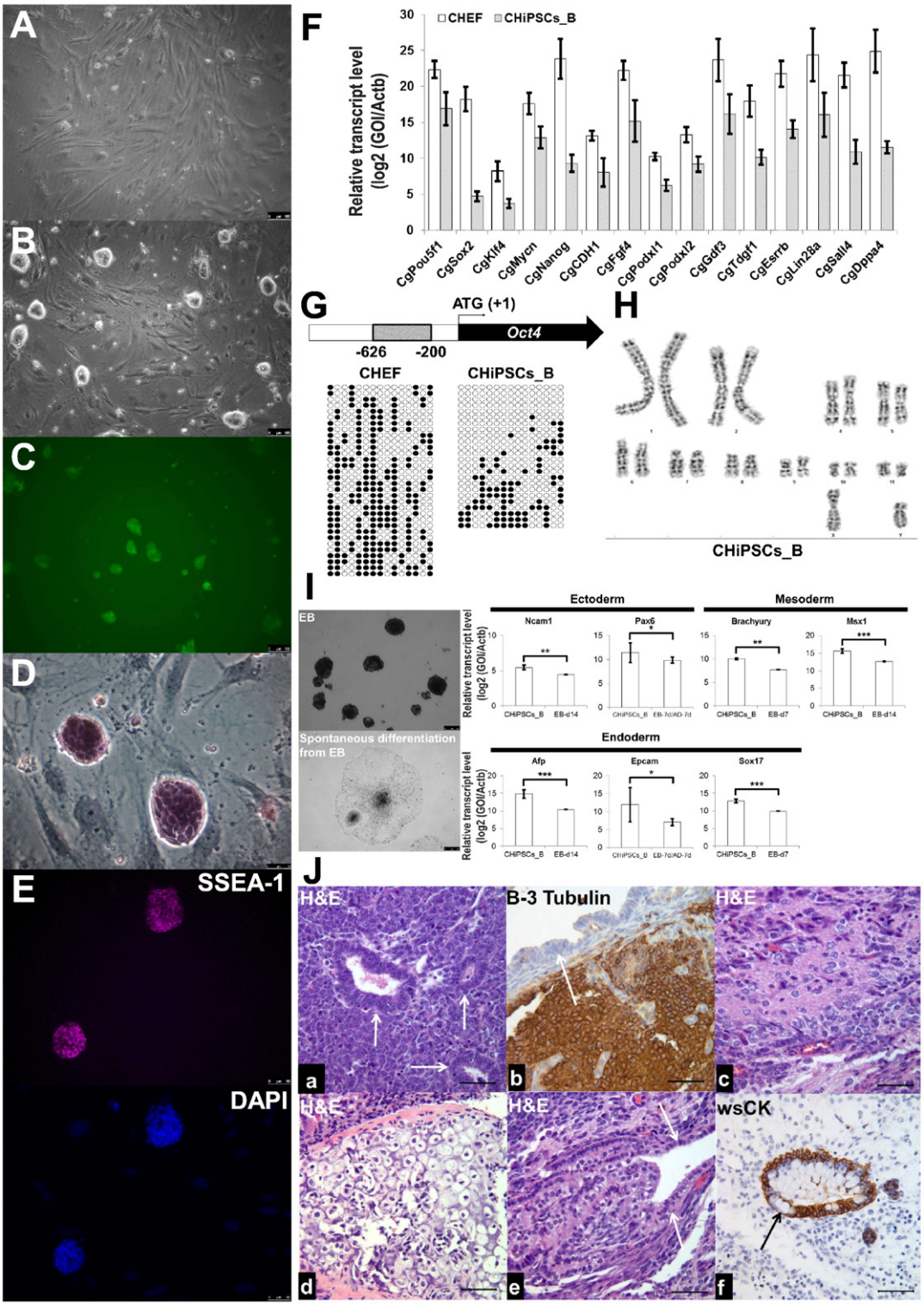

Fig. 1. Characterization of Chinese hamster-induced pluripotent stem cells (CHiPSCs) generated by lentiviral vector transduction. (A) Chinese hamster embryonic fibroblasts (CHEF). (B) Emergence of colonies. (C) Nanog-promopter GFP lentivirus infected cells of clone B. (D) AP staining of a colony from clone B. (E) Immunostaining of clone B for SSEA-1 and DAPI. Scale bar: 100 μm for A, B, and C; 25 μm for D; 50 μm for E. (F) Quantitative reverse transcription-PCR analyses of endogenous pluripotent marker transcripts in CHiPSCs-B. Data represented as mean ± SEM. $p < 0.001$. Cg in the label denotes that probes for the endogenous (Chinese hamster) gene were used. (G) Bisulfite genomic sequencing of the promoter region of Oct4. The open and closed circles represent a CpG site, representing unmethylated and methylated status respectively. (H) The CHiPSCs-B showed a normal 22,XY karyotype. (I) EB formed by CHiPSCs-B and the EB spreading on matrigel 7 days after plating for differentiation preceded by another 7 days of EB formation (Scale bar: 250 μm). qRT-PCR analyses of transcripts of differentiation markers of the three germ layers (Ncam1, Pax6, Brachyury (T), Msx1, Afp, Epcam and Sox17). Actin (Actb) transcript level was used as the reference. Data represented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Histology of teratomas derived from CHiPSCs-B on (Ja) neurectodermal tissues comprised the majority of the teratoma and consisted of primitive neural tissues characterized by formation of neural rosettes (arrows) and cords of small polyhedral cells (surrounding rosettes) typical of neural progenitors which were (Jb) positive for β-3 tubulin (dark brown, IHC stain). (Jc) Other regions showed more mature central nervous system-like structures. (Jd) Multiple sites of cartilage differentiation were present which represent a mesenchymal lineage. (Je) Endodermal differentiation was demonstrated by the presence of tubular structures which were lined by cuboidal to columnar epithelial cells (arrows) and which were (Jf) positive for wide-spectrum cytokeratins (wsCK). The epithelium also contained cells with clear vacuoles consistent with mucus-containing goblet cells (arrow). Also noted was the absence of β-3 tubulin immunoreactivity in cells as seen in (Jb, arrow). Scale bar: 50 μm.