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Lab Resource: Stem Cell Line

Generation of iPSC line iPSC-FH2.1 in hypoxic conditions from human foreskin fibroblasts

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ABSTRACT

Human foreskin fibroblasts were used to generate the iPSC line iPSC-FH2.1 using the EF1a-hSTEMCCA-loxP vector expressing OCT4, SOX2, c-MYC and KLF4, in 5% O_2 culture conditions. Stemness was confirmed, as was pluripotency both *in vivo* and *in vitro*, in normoxia and hypoxia. Human Embryonic Stem Cell (hESC) line WA-09 and reprogrammed fibroblast primary culture HFF-FM were used as controls.

Resource table

Name of stem cell line	iPSC-FH2.1
Institution	LIAN-CONICET, FLENI Laboratorio de Regulación de la Expresión Génica en Células Madre, Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires
Person who created resource	María Questa
Contact person and email	mariaquesta@gmail.com
Date archived/stock date	2010
Origin	Human foreskin fibroblasts
Type of resource	Biological reagent: induced pluripotent stem cell (iPS); derived from human healthy fibroblasts, in 5% O_2
Sub-type	Cell line
Key transcription factors	Oct4, Sox2, cMyc, Klf4
Authentication	Identity and purity of cell line confirmed (Figs. 1 and 2)
Link to related literature	None
Information in public databases	No
Ethics	Patient informed consent obtained Ethics Review Board approval obtained

Resource details

We obtained fibroblasts from a healthy donor (primary culture HFF-FM) and reprogrammed them using the STEMCCA vector to generate the iPSC-FH2.1 line, in hypoxic conditions. Control lines iPSC-FN2.1 and iPS6.1 were generated in parallel, in normoxia, showing the same results as iPSC-FH2.1, regarding their identity and purity. The iPSC-FH2.1 line exhibited classic pluripotent stem cell morphology: small cells with low nucleus:cytoplasm ratio, organized in colonies with defined borders that grew as three-dimensional structures (Fig. 1A). As assessed by RT-qPCR, the expression of stemness markers *POU5F1*, *NANOG* and *C-MYC* was induced when cells were reprogrammed, compared to donor fibroblasts (Fig. 1B). The expression of several human stemness markers, including NANOG, POU5F1, SOX2, SSEA-4, TRA1–60, TRA-1–81 and C-MYC, was also studied by immunostaining (Fig. 1C), exhibiting clear expression of these markers when cultured in normoxic and hypoxic conditions.

To assess if reprogramming had any effect on promoter DNA modifications, we studied the methylation of the *POU5F1* promoter (Fig. 1D). We found that on a global level the *POU5F1* promoter DNA region is demethylated on several CpG islands in pluripotent cells; whereas in fibroblasts that originated them the promoter is widely methylated. This supports the occurrence of a profound remodeling of gene expression.

The transgene inserted by the STEMCCA lentiviral vector was reported to become silenced after the reprogramming event (Sommer et al., 2009). In order to study the endogenous expression of the endogenour genes (as in Fig. 1A), we needed to be sure that the expression of the exogenous genes carried in the vector had been silenced and we could,

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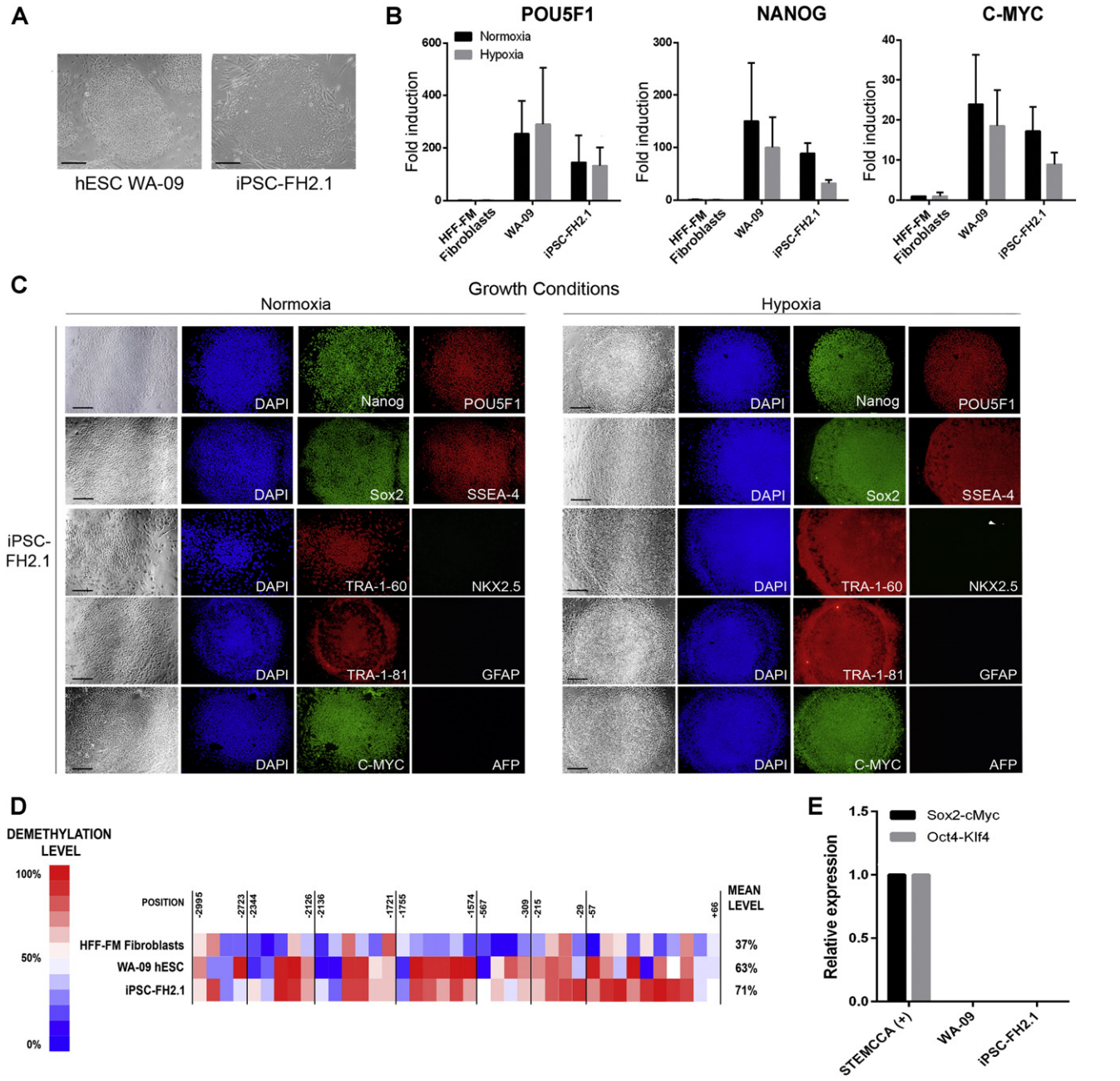

Fig. 1. iPSC-FH2.1 iPSC line's stemness. Description of data: (A) Phase contrast image of representative colonies cultured in normoxia. Scale bars: 200 μm. (B) RT-qPCR analysis of expression of stemness markers *POU5F1*, *NANOG* and *C-MYC*, in cells cultured either in normoxia or hypoxia for 72 h. Fold-induction *vs.* control is shown in bars representing the mean ± SEM of three independent experiments. Inductions marked with * are significant with respect to HFF-FM Fibroblasts in the same O_2 condition, with $p < 0.05$. (C) Representative phase contrast and immunostaining images for stemness markers, in normoxia and chronic hypoxia. Nuclei were stained with DAPI. Scale bars: 200 μm. (D) Demethylation levels of the *POU5F1* promoter. Each square represents a CpG and each section delimited by a black line represents an amplicon of PCR. The numbers indicate the base positions relative to the transcription start site. (E) RT-qPCR analysis of the expression of the transgenes carried by the lentiviral reprogramming vector. STEMCCA is the vector itself used as a positive control. Fold-induction *vs.* control is shown in bars representing the mean ± SEM of three independent experiments. Repression for hESC and iPSC line is significant with $p < 0.05$.

therefore, measure only endogenous expression from cDNA (Fig. 1E). There is no detectable expression of the transgenes, as studied by RT-qPCR on established iPSC lines, using primers specific for the exogenous expression of the lentiviral genes.

Stemness markers that are induced during reprogramming are down-regulated during differentiation. The stem cell line was induced to undergo differentiation in normoxia and *POU5F1*, *SOX2* and *NANOG* expression decreased between day 0 (undifferentiated state) and day 21 of the differentiation process (Fig. 2A). Moreover, germ layer lineage marker expression was induced during the differentiation when cells were cultured both in normoxia or hypoxia for a long-term period (Fig. 2B). Germ layer markers were all induced from a basal expression at day 0 to an increased expression at day 7 or day 21, depending on the kinetics of each gene. All the cell lines were able to differentiate *in vitro* to the three germ layers. The presence of differentiation marker proteins was also observed by immunostaining (Fig. 2C), both when grown in normoxia or hypoxia. The iPSC lines established also showed pluripotency *in vivo*, being able to generate teratomas in immunosuppressed mice (Fig. 2D). The teratomas generated had cells of the three germ layers, which could be assigned to different tissues and structures. Finally, we found that all the cell lines generated and studied possessed a normal human karyotype, and there were no significant genetic anomalies introduced by the reprogramming process (Fig. 2E).