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Lab resource: Stem cell line

Episomal plasmid-based generation of induced pluripotent stem cells from fetal femur-derived human mesenchymal stromal cells

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ABSTRACT

Human bone mesenchymal stromal cells derived from fetal femur 55 days post-conception were reprogrammed to induced pluripotent stem cells using episomal plasmid-based expression of *OCT4*, *SOX2*, *NANOG*, *LIN28*, *SV40LT*, *KLF4* and *c-MYC* and supplemented with the following pathway inhibitors — TGFβ receptor inhibitor (A-83-01), MEK inhibitor (PD325901), GSK3β inhibitor (CHIR99021) and ROCK inhibitor (HA-100). Successful induction of pluripotency in two iPS-cell lines was demonstrated in vitro and by the Pluritest.

Resource table.

Name of stem cell construct	pEP4 E02S EN2K (Addgene, #20925), pCEP4-M2L (Addgene, #20926), pEP4 E02S ET2K (Addgene, #20927)
Institution	Max Planck-Institute for Molecular Genetics
Person who created resource	Matthias Megges
Contact person and email	James Adjaye, James.Adjaye@med.uni-duesseldorf.de
Date archived/stock date	May 22, 2012
Origin	Primary human mesenchymal stromal cells isolated from fetal femur 55 days post-conception
Type of resource	Biological reagent: induced pluripotent stem cell (iPS-cell); generated from primary human mesenchymal stromal cells isolated from the fetal femur 55 days post-conception
Sub-type	Cell line
Key transcription factors	OCT4, SOX2, NANOG, LIN28, SV40LT, KLF4 and c-MYC
Authentication	Identity of cell line confirmed (Fig. 1 and Fig. 2)
Link to related literature (direct URL links and full references)	http://onlinelibrary.wiley.com/doi/10.1634/stemcells.2005-0368/pdf
Information in public databases	No

1. Resource details

Episomal plasmids harboring the transgenes *OCT4*, *SOX2*, *NANOG*, *LIN28*, *SV40LT*, *KLF4* and *c-MYC* were introduced into human mesenchymal stromal cells derived from the fetal femur of the age 55 days post-conception (fetal hMSCs) by nucleofection. In addition, a combination of TGFβ receptor inhibitor — A-83-01, MEK inhibitor — PD325901, GSK3β inhibitor — CHIR99021 and ROCK inhibitor — HA-100 was used to enhance the efficiency of reprogramming (Yu et al., 2011). Two iPS-cell lines, MSC-iPS(fetal)#1 and MSC-iPS(fetal)#2 were characterized. The expression of pluripotency-associated markers was confirmed by immunofluorescence staining and microarray-based transcriptome profiling (Fig. 1A and B). The transcriptomes of MSC-iPS(fetal)#1 and MSC-iPS(fetal)#2 were more comparable to the transcriptome of the human embryonic stem cell line H1 (hESC H1) than to that of their parental cells with Pearson correlations of 0.953 and 0.952 respectively (Fig. 1C and D). In addition, the origin of the cell-types as well as the absence of episomal plasmids in the generated iPS cells was confirmed by PCR (Fig. 1D and E). Chromosome analyses of iPS(fetal)#1 and MSC-iPS(fetal)#2 revealed a normal male karyotype for both iPS-cell lines (Fig. 2A). Pluripotency in vitro was also confirmed by embryoid body-based assays (Fig. 2B). Both iPS-cell lines showed high transcriptome similarity with hESC H1 compared to the parental cells based on the in silico PluriTest (Fig. 2C) (Müller et al., 2011).

2. Materials and methods

2.1. Ethics statement

Fetal femur cells were derived from fetal tissue obtained at 55 days post-conception following informed, written patient consent obtained at the termination of pregnancy. Approval was obtained by the

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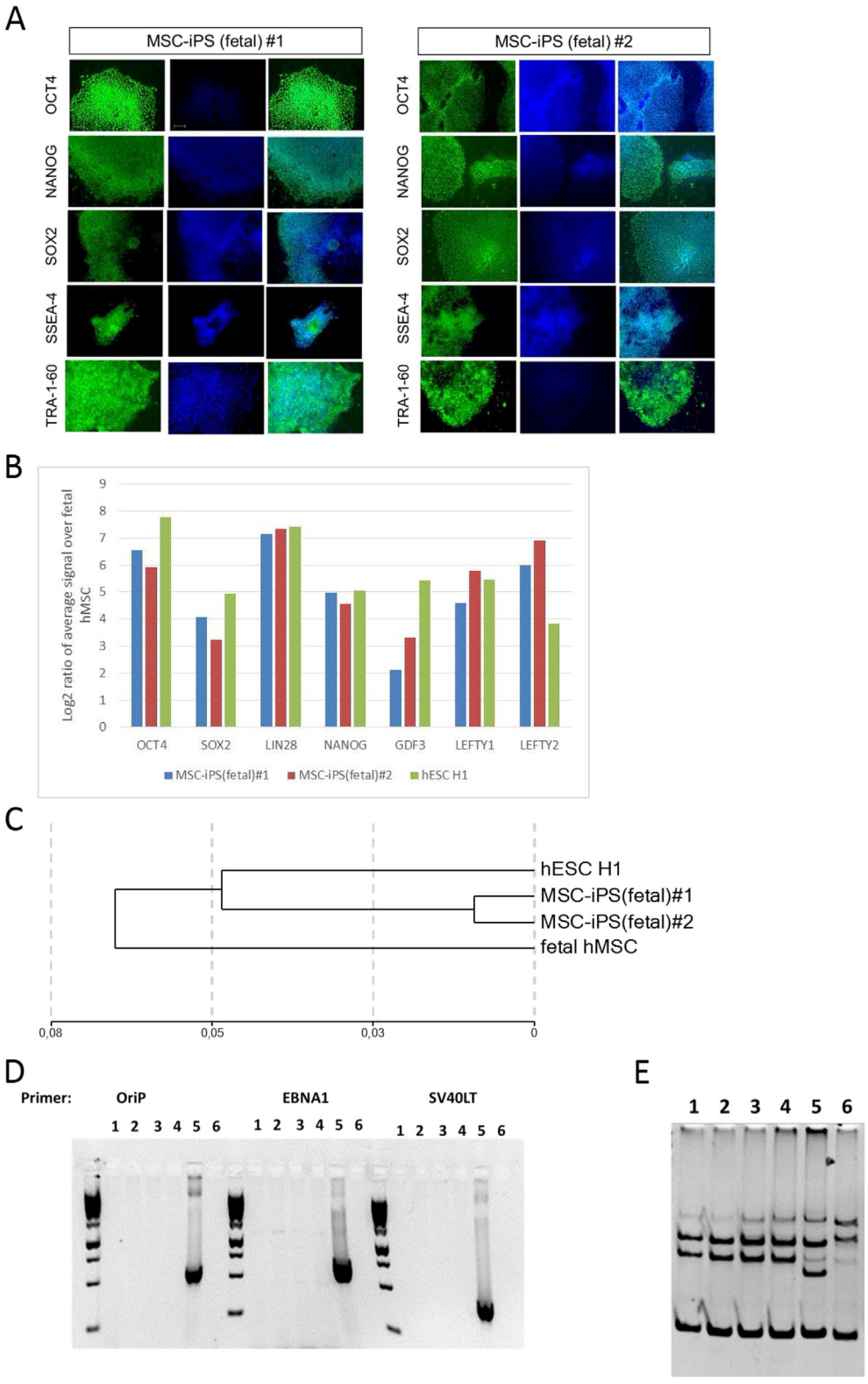

Fig. 1. Confirmation of pluripotency marker expression, absence of episomal plasmids and proof of the parental cell type. A) Expression of pluripotency-associated markers in MSC-iPS(fetal)#1 and MSC-iPS(fetal)#2 confirmed by immunofluorescence staining. B) mRNA expression derived from the microarray-based transcriptome profiling dataset. The diagram shows the Log2 ratio of the average signal detected in the respective iPS-cell line or hESC H1 as control over the average signal detected in parental fetal hMSCs. C) Correlation values between the transcriptomes of MSC-iPS(fetal)#1, MSC-iPS(fetal)#2, their parental primary hMSCs and hESC H1 based on expression values detected by microarray. Pearson correlation is shown between the samples calculated with the software GenomeStudio (Illumina). D) Clustering dendrogram based on Pearson correlation depicting the similarity between parental fetal hMSCs and the generated iPS-cell lines and hESC H1. The dendrogram was generated using the software Genome Studio (Illumina). E) DNA-fingerprinting to confirm the origin of MSC-iPS(fetal)#1 and MSC-iPS(fetal)#2. Polymerase chain reaction with a microsatellite-specific primer. Acrylamide gel electrophoresis. Samples: 1: parental fetal hMSC; 2: MSC-iPS (fetal)#1; 3: MSC-iPS(fetal)#2; 4: iPS-cell line derived from fetal hMSC, not part of this study; 5: hESC H1; 6: hESC H9.). F) Confirmation of the absence of the episomal plasmids used for reprogramming. Polymerase chain reaction using primer specific for *OriP*, *EBNA1* or *SV40LT* on the episomal plasmids. Genomic DNA was used as template. hESC H1 and hESC H9 were analyzed in parallel to rule out cross contamination. Samples: 1: parental fetal hMSC; 2: iPS-cell line derived from fetal hMSC, not part of this study; 3: MSC-iPS(fetal)#1; 4: MSC-iPS(fetal)#2; 5: episomal plasmid DNA of pEP4 E02S ET2K; 6: no template. Size marker: GeneRuler 1 kb DNA Ladder (Life Technologies).