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Stem Cell Research: Lab Resource

Generation of iPSC from cardiac and tail-tip fibroblasts derived from a second heart field reporter mouse

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ABSTRACT

Mef2c Anterior Heart Field (AHF) enhancer is activated during embryonic heart development and it is expressed in multipotent cardiovascular progenitors (CVP) giving rise to endothelial and myocardial components of the outflow tract, right ventricle and ventricular septum. Here we have generated iPSC from transgenic Mef2c-AHF-Cre x Ai6(RCLZsGreen) mice. These iPSC will provide a novel tool to investigate the AHF-CVP and their cell progeny.

Resource Table

Name of Stem Cell line	AHFiPS7 and AHFiPS19
Institution	Cell Therapy Program, Foundation for Applied Medical Research (FIMA), University of Navarra.
Person who created resource	Xonia Carvajal-Vergara, Estibaliz Arellano-Viera, Javier Linares
Contact person and email	xcarvajal@unav.es; fprosper@unav.es
Date archived/stock date	March 2015
Origin	Mouse tail-tip fibroblasts and mouse cardiac fibroblasts
Type of resource	Biological reagent: mouse induced pluripotent stem cell (iPSC) lines derived from transgenic Mef2c-AHF-Cre x Ai6(RCLZsGreen) mice
Sub-type	Mouse induced pluripotent stem cell (iPSC) lines
Key transcription factors	Oct4, Sox2, cMyc, Klf4
Authentication	Identity and purity of cell line confirmed (Figs. 1 and 2)
Link to related literature	Not available
Information in public databases	Not available

Resource Details

Tail-tip fibroblasts (TTF) and cardiac fibroblasts (CF) derived from Mef2c-AHF-Cre (Verzi et al., 2005) x Ai6(RCLZsGreen) (Madisen et al., 2010) mice were transduced with Oct4, Sox2, Klf4 and c-Myc (OSKM) with the pMXs retrovirus vectors. Mouse ES-like colonies appeared 15–20 days after transduction and were picked and seeded on irradiated mouse embryonic fibroblasts (γMEFs) and maintained with LIF. Established iPSC, AHF-iPSC, derived from TTF (AHFiPS7) and CF (AHFiPS19) encoded the expected genomic insertions (Fig. 1a). The karyotypes of AHF-iPSC were normal (Fig. 1b) and the transgenes were silenced in established AHF-iPSC lines as shown by qRT-PCR (Fig. 1c). Endogenous pluripotency-associated markers such Oct4, Sox2, Nanog and Zfp42 were expressed in AHF-iPSC, analyzed by qRT-PCR (Fig. 1d), and Nanog (Fig. 1e) and alkaline phosphatase (AP, Fig. 1f) expression was verified by cell staining. To demonstrate the capacity of AHF-iPSC lines to differentiate into the three germ layers we performed in vivo teratoma and in vitro embryoid body (EB) differentiation assays. Teratomas contained tissues derived from the three germ layers (Fig. 2a). We collected RNA from undifferentiated AHF-iPSC and from EB on day 7 and 14 of differentiation. Increased expression of Cxcl12/Mash1 (ectoderm), Acta2/Myh6 (mesoderm) and Hnf4a/Afp (endoderm), (Fig. 2b) was observed. Mef2c and ZsGreen were not detected in undifferentiated AHF-iPSC, however, the expression of Mef2c and ZsGreen was confirmed by qRT-PCR on day 7 and 14 of differentiation (Fig. 2b), and ZsGreen was observed under fluorescence microscopy (Fig. 2c).

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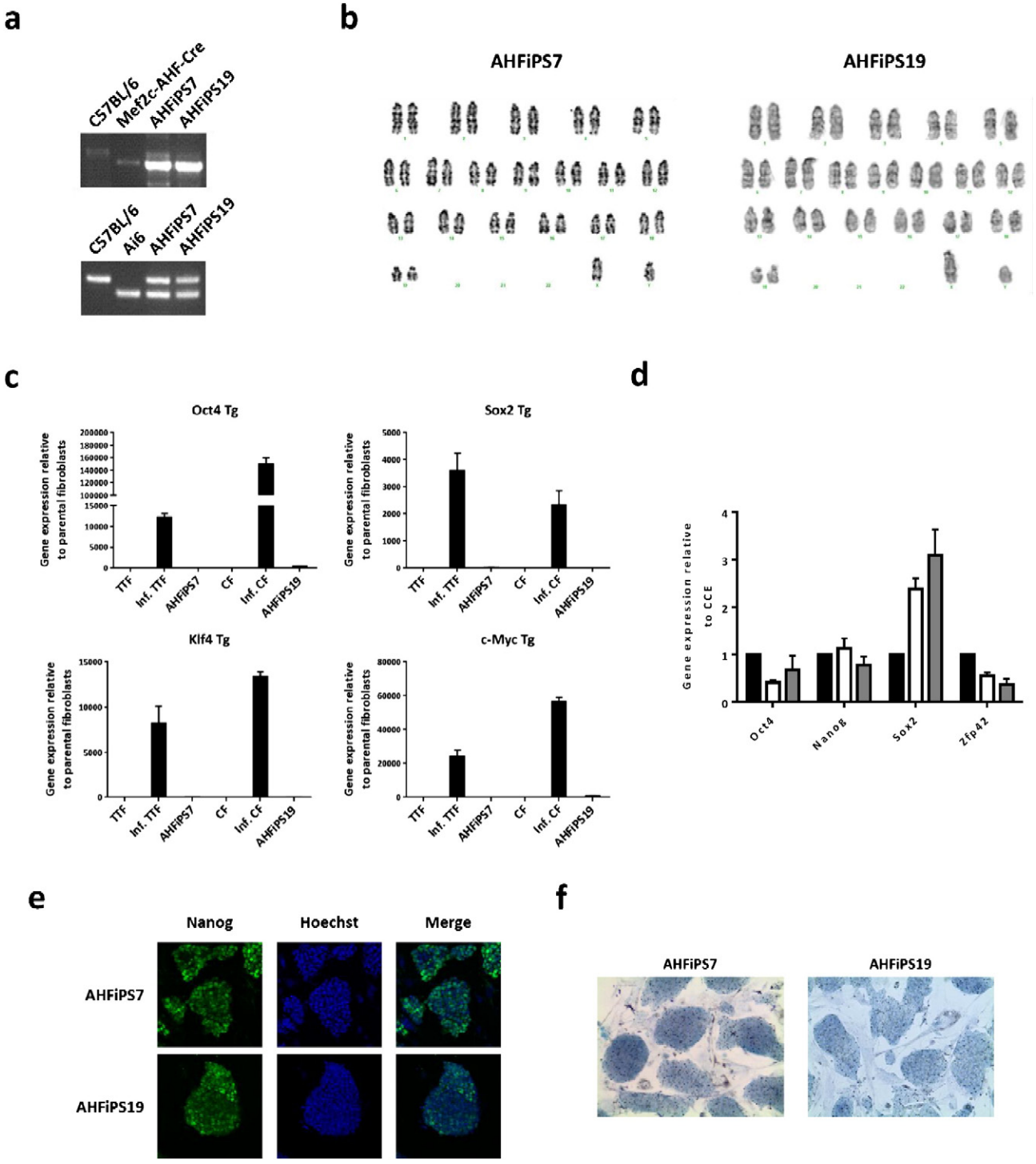

Fig. 1. Characterization of AHFiPS7 and AHFiPS19 cell lines. a) AHFiPS7 and AHFiPS19 encode the expected knock in sequences in ROSA and AHF loci. Samples from wild-type C57BL/6, and Ai6 and Mef2c-AHF-Cre mice were used as negative and positive controls, respectively. **b**) Karyotype analysis of AHFiPS7 and AHFiPS19. Normal karyotypes of 40, XY. **c**) Transgene (Tg) silencing of Oct4, Sox2, Klf4 and c-Myc in the established AHF-iPSC lines was verified by qRT-PCR after passage 9. Infected fibroblast samples were used as positive control of the Tg expression. **d**) Gene expression analysis by qRT-PCR of the endogenous pluripotency-associated markers Oct4, Sox2, Nanog and Zfp42. Black bars: CCE; white bars: AHFiPS7; grey bars: AHFiPS19. **e**) Immunostaining against Nanog in AHFiPS7, AHFiPS19. Nuclei: Hoescht staining. **f)** Alkaline phosphatase enzymatic activity of AHFiPS7 and AHFiPS19.

Fig. 2. Differentiation potential of AHFiPS7 and AHFiPS19 cell lines. a) In vivo differentiation: teratoma formation assay. The pictures show H&E staining with representative tissues from the three germ layers. Neural rosettes (left), cartilage tissue and muscle (middle), and endodermal epitheliums (right). **b)** In vitro differentiation: EB differentiation assay. Gene expression analysis by qRT-PCR of ZsGreen and Mef2c, and markers of ectoderm (Cxcl12 and Mash1), mesoderm (Acta2 and Myh6), and endoderm (Hnf4a and Afp). Solid lines: AHFiPS7; dashed lines: AHFiPS19. **c)** ZsGreen expression was analyzed by fluorescence microscopy in undifferentiated AHF-iPSC and in EB on day 7 and 14 of differentiation. Scale bar, 100 μm.