Generation of fibrodysplasia ossificans progressiva and control integration free iPSC lines from periodontal ligament fibroblasts


Published in:
Stem Cell Research

DOI:
10.1016/j.scr.2019.101639

Link to publication

Creative Commons License (see https://creativecommons.org/use-remix/cc-licenses):
CC BY-NC-ND

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (http://dare.uva.nl)
# Generation of Fibrodysplasia ossificans progressiva and control integration free iPSC lines from periodontal ligament fibroblasts

G. Sanchez-Duffhues⁴,⁵,⁎, H. Mikkers⁶,⁷, D. de Jong⁸, K. Szuha⁹, T.J. de Vries¹, C. Freund³,⁴, N. Bravenboer³, R.J.J. van Es¹, J.C. Netelenbos⁸, M.-J. Goumans², E.M.W. Eekhoff⁸, P. ten Dijke⁴

**Abstract**

Fibrodysplasia ossificans progressiva (FOP) is a very rare devastating heterotopic ossification disorder, classically caused by a heterozygous single point mutation (c.617G>A) in the ACVR1 gene, encoding the Bone morphogenetic protein (BMP) type I receptor, also termed activin receptor-like kinase (ALK)2. FOP patients develop heterotopic ossification episodically in response to inflammatory insults, thereby compromising tissue sampling and the development of in vitro surrogate models for FOP. Here we describe the generation and characterization of a control and a classical FOP induced pluripotent stem cell (iPSC) line derived from periodontal ligament fibroblast cells using Sendai virus vectors.

<table>
<thead>
<tr>
<th>Unique stem cell lines identifier</th>
<th>LUMCi009-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternative names of stem cell lines</td>
<td>LUMCi010-A</td>
</tr>
<tr>
<td>Institution</td>
<td>Leiden University Medical Center</td>
</tr>
<tr>
<td>Contact information of distributor</td>
<td>Prof. Peter ten Dijke, <a href="mailto:P.ten_Dijke@lumc.nl">P.ten_Dijke@lumc.nl</a></td>
</tr>
<tr>
<td>Type of cell lines</td>
<td>iPSC</td>
</tr>
<tr>
<td>Origin</td>
<td>Human</td>
</tr>
<tr>
<td>Cell Source</td>
<td>Periodontal ligament fibroblast</td>
</tr>
<tr>
<td>Clonality</td>
<td>Clonal</td>
</tr>
<tr>
<td>Method of reprogramming</td>
<td>Integration free Sendai virus</td>
</tr>
<tr>
<td>Multiline rationale</td>
<td>Control and disease pair</td>
</tr>
<tr>
<td>Gene modification</td>
<td>YES</td>
</tr>
<tr>
<td>Type of modification</td>
<td>Spontaneous mutation</td>
</tr>
<tr>
<td>Associated disease</td>
<td>Fibrodysplasia ossificans progressiva</td>
</tr>
<tr>
<td>Gene/locus</td>
<td>c.617G4A; p. (Arg206His)</td>
</tr>
<tr>
<td>Method of modification</td>
<td>N/A</td>
</tr>
<tr>
<td>Name of transgene or resistance</td>
<td>N/A</td>
</tr>
<tr>
<td>Inducible/constitutive system</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Date archived/stock date: 30/12/2015
Cell line repository/bank: N/A
Ethical approval: The study has been approved by the Vrije Universiteit Medisch Centrum (VUMC) Amsterdam Research Committee, with protocol number 2012.467

1. Resource utility

Patients with Fibrodysplasia ossificans progressiva (FOP) develop bone formation at extra skeletal sites in response to trauma. Therefore, induced pluripotent stem cells (iPSCs) generation may be useful to establish patient-derived primary cell lines in order to further understand the specific pathophysiological mechanisms induced by ALK2 R206H causing ectopic bone formation.

2. Resource details

Fibrodysplasia ossificans progressiva (FOP) is an extremely rare congenital disease (1 in 2,000,000 individuals) characterized by a heterozygous point mutation in the ACVR1gene, encoding the Bone morphogenetic protein type I receptor, also termed activin receptor-like kinase (ALK)2. The most common ACVR1 mutation in approximately 98% of all FOP patients c.617G->A causes the amino acid substitution
R206H in the glycine serine rich intracellular domain of ALK2 (Shore et al., 2006). Here we present an iPSC line generated from periodontal ligament fibroblasts from a female patient with FOP, and a control line from a healthy donor. Periodontal ligament fibroblasts were collected from a 23 years old female with classical FOP, diagnosed with a trismus and pericoronitis of a lower wisdom tooth (Eekhoff et al., 2018), and a 30 years old control female, following the same surgical intervention to remove a wisdom tooth.

Periodontal ligament fibroblasts (PDLs), the cells that enable the anchoring of teeth into bone, were cultured from the donated biopsy samples and were frozen in liquid nitrogen at passage 3. As previously shown, periodontal ligament cells from FOP can be used to address both osteogenesis and osteoclastogenesis aspects of the disease (de Vries et al., 2018). Primary cells however have a limited life span, therefore, iPSC FOP cell models are desired. Reprogramming was performed using a Sendai virus vector containing MYC, KLF4, SOX2 and OCT4, using the vector published by Nishimura et al. (2011), and clonal iPSC lines (LUMCi009-A, LUMCi010-A) were established and characterized (Fig. 1A) (Table 1). The pluripotent nature of the cells was assessed by immunofluorescent staining with specific antibodies against Nanog, Oct4 and SSEA-4 (Fig.1B), and the expression of the pluripotent gene markers SOX2, OCT3/4, RONINand REX1 by quantitative rtPCR (Supp.)

Figure 1. Characterization of the IPS cell lines LUMC0085 and LUMC0084.
iPSC clones were passaged at 1:10 (ThermoFisher Scientific). PDLC fibroblasts 36 h after transduction with Sendai particles were used as positive control. Cell line authentication using profiling of 23 STR loci demonstrated that parental and iPSC-derived lines are identical (data not shown). Multicolour FISH based molecular karyotyping was performed at cell level to detect numerical changes, interchromosomal exchanges (translocation, insertion) and large deletions, and pericentric inversions. This analysis did not reveal any large genomic aberrations and confirmed that the iPSC lines were female (46, XX) (Fig. 1C). Using Sanger sequencing we demonstrated the absence and presence of the classical FOP mutation (c.617G>A) in the ACVR1R exon 4 in control and FOP PDLC fibroblasts and iPSC lines, respectively (Fig.1D). Finally, the potential of the LUMC009-A, LUMCi010-A iPSC lines to give rise to the three germ layers was demonstrated by their spontaneous in vitro differentiation into mesoderm (CD31), ectoderm (βIII-tubulin) and endoderm (AFP) derivatives (Fig. 1E). All cell lines generated were negative for mycoplasma. A summary of the characterization of LUMC0084 and LUMC0085 is shown in Table 2.

### 3. Materials and methods

#### 3.1. Cell culture and reprogramming

Periodal ligament fibroblast (PDL) cells were cultured as reported before (de Vries et al., 2018). At passage 6 1.10^5 cells were transduced with Sendai virus (SeVdp(KOSM)302L) at a multiplicity of infection of 10. After 2 days, 15,000 transduced cells were seeded onto a fresh layer of irradiated CD1 mouse embryonic fibroblasts (MEFs). From day 3 cells were cultured in HESC medium (DMEM/F12 (ThermoFisher Scientific) with 20% knockout serum replacement (KSR) (ThermoFisher Scientific), 10 ng/ml bFGF (Peprotech), 100 μM β-mercaptoethanol, 10 μg/ml ascorbic acid (Sigma), GlutaMax (ThermoFisher Scientific), 1% Penicillin-Streptomycin (ThermoFisher Scientific), 1% non-essential amino acids (NEAA) (ThermoFisher Scientific) and with (FOP cells) or without (control cells) 1 μM LDN-193,189 (Sigma-Aldrich) at day 8. Around week 3 visible iPSC colonies were manually transferred into a Vitronectin (StemCell Technologies)-coated 6 well plate in TESR-E8 (StemCell Technologies) at 37 °C with 5% CO2. iPSCs were fixed with 2% paraformaldehyde (PFA) for 30 min at room temperature (RT), washed with 0.1 M glycine, permeabilized with 0.1% Triton X-100 and blocked in phosphate buffer saline (PBS) containing 4% normal swine serum (NSS) for one hour. Next, the cells were incubated overnight at 4 °C in blocking solution containing primary antibody. Next day, the cells were washed in PBS and incubated with secondary antibody for one hour at RT. Finally, the cells were washed and mounted in Prolong Gold containing DAPI (Invitrogen). The preparations were imaged with a Leica SP5 confocal scanning laser microscope. Antibodies are described in Table 3.

#### 3.2. Immunofluorescent staining

iPSCs were fixed with 2% paraformaldehyde (PFA) for 30 min at room temperature (RT), washed with 0.1 M glycine, permeabilized with 0.1% Triton X-100 and blocked in phosphate buffered saline (PBS) containing 4% normal swine serum (NSS) for one hour. Next, the cells were incubated overnight at 4 °C in blocking solution containing primary antibody. Next day, the cells were washed in PBS and incubated with secondary antibody for one hour at RT. Finally, the cells were washed and mounted in Prolong Gold containing DAPI (Invitrogen). The preparations were imaged with a Leica SP5 confocal scanning laser microscope. Antibodies are described in Table 3.

#### 3.3. Quantitative real-time PCR analysis

Total RNA extraction was performed using NucleoSpin RNA II (Machery Nagel). 500 ng of RNA were retro-transcribed using RevertAid First Strand cDNA Synthesis Kits (Fisher Scientific), and real-time reverse transcription-PCR experiments were performed using SYBR Green (Bio-Rad) and a Bio-Rad CFX Connect device. Oligonucleotides are shown in Table 3.

#### 3.4. Mycoplasma detection

The absence of mycoplasma was tested using the MycoAlert mycoplasma detection kit (Lonza) according to the manufacturer’s instructions.

#### 3.5. ACVR1 mutation analysis

100 ng of DNA were subjected to PCR to amplify the exon 4 of ACVR1/ALK2, as reported before (Shore et al., 2006). The PCR product was separated in a 1% agarose gel, purified and submitted to Sanger sequencing. Oligonucleotides used for sequencing are described in Table 3.

#### 3.6. Human cell line authentication

The human cell lines listed below have been tested by means of the Cell Dissociation Reagent (StemCell Technologies).

### Table 1

<table>
<thead>
<tr>
<th>iPSC line names</th>
<th>Abbreviation in figures</th>
<th>Gender</th>
<th>Age</th>
<th>Ethnicity</th>
<th>Genotype of locus</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>LUMC008SicTRL</td>
<td>LUMC0085</td>
<td>Female</td>
<td>30</td>
<td>Caucasian</td>
<td>c.617G</td>
<td>N/A</td>
</tr>
<tr>
<td>LUMC0084iFOP</td>
<td>LUMC0084</td>
<td>Female</td>
<td>23</td>
<td>Caucasian</td>
<td>c.617A</td>
<td>Fibrodysplasia ossificans progressiva</td>
</tr>
</tbody>
</table>

---

3.2. Immunofluorescent staining

iPSCs were fixed with 2% paraformaldehyde (PFA) for 30 min at room temperature (RT), washed with 0.1 M glycine, permeabilized with 0.1% Triton X-100 and blocked in phosphate buffered saline (PBS) containing 4% normal swine serum (NSS) for one hour. Next, the cells were incubated overnight at 4 °C in blocking solution containing primary antibody. Next day, the cells were washed in PBS and incubated with secondary antibody for one hour at RT. Finally, the cells were washed and mounted in Prolong Gold containing DAPI (Invitrogen). The preparations were imaged with a Leica SP5 confocal scanning laser microscope. Antibodies are described in Table 3.

#### 3.3. Quantitative real-time PCR analysis

Total RNA extraction was performed using NucleoSpin RNA II (Machery Nagel). 500 ng of RNA were retro-transcribed using RevertAid First Strand cDNA Synthesis Kits (Fisher Scientific), and real-time reverse transcription-PCR experiments were performed using SYBR Green (Bio-Rad) and a Bio-Rad CFX Connect device. Oligonucleotides are shown in Table 3.

#### 3.4. Mycoplasma detection

The absence of mycoplasma was tested using the MycoAlert mycoplasma detection kit (Lonza) according to the manufacturer's instructions.

#### 3.5. ACVR1 mutation analysis

100 ng of DNA were subjected to PCR to amplify the exon 4 of ACVR1/ALK2, as reported before (Shore et al., 2006). The PCR product was separated in a 1% agarose gel, purified and submitted to Sanger sequencing. Oligonucleotides used for sequencing are described in Table 3.

#### 3.6. Human cell line authentication

The human cell lines listed below have been tested by means of the
The authors have no conflicts of interest to declare.

**Supplementary materials**

Supplementary material associated with this article can be found, in the online version, at 10.1016/j.scr.2019.101639.

**References**


de Vries, T.J., Schoenmaker, T., Michi, D., Heegrovst, J., Bouwkla, S., Forouzanfar, T., Pals, G., Netelenbos, C., Eckhoff, E.M.W., Bravenboer, N., 2018. Periodontal ligament fibroblasts (HpdIF Cont and HpdIF FOP, respectively) and control and FOP iPSC lines (iPSC Cont and iPSC FOP, respectively) was performed and is available as supplementary material. Technical details were uploaded alongside the STR analysis results.

**Declaration of Competing Interest**

The authors have no conflicts of interest to declare.

**Supplementary materials**

Supplementary material associated with this article can be found, in the online version, at 10.1016/j.scr.2019.101639.

**References**


