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Lab resource: Stem cell line

Generation of a human iPSC line from a patient with a mitochondrial encephalopathy due to mutations in the *GFM1* gene

Francisco Zurita-Díaz [a,b,c], Teresa Galera-Monge [a,b,c], Ana Moreno-Izquierdo [c,d], Mario F. Fraga [e], C. Ayuso [b,f], Agustin F. Fernández [g], Rafael Garesse [a,b,c], M. Esther Gallardo [a,b,c,*]

[a] Departamento de Bioquímica, Instituto de Investigaciones Biomédicas "Alberto Sols ", Facultad de Medicina (UAM-CSIC), Spain
[b] Centro de Investigación Biomédica en Red en Enfermedades Raras (CIBERER) Madrid, Spain
[c] Instituto de Investigación Hospital 12 de Octubre ("i + 12"), Madrid, Spain
[d] Servicio de Genética, Hospital 12 de Octubre, Madrid, Spain
[e] Nanomaterials and Nanotechnology Research Center (CINN-CSIC), Spain
[f] Department of Genetics, Instituto de Investigación Sanitaria-University Hospital Fundacion Jimenez Diaz (IIS-FJD, UAM), Madrid, Spain
[g] Cancer Epigenetics Laboratory, Institute of Oncology of Asturias (IUOPA), HUCA, Universidad de Oviedo, Oviedo, Spain

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ABSTRACT

Human iPSC line GFM1SV.25 was generated from fibroblasts of a child with a severe mitochondrial encephalopathy associated with mutations in the *GFM1* gene, encoding the mitochondrial translation elongation factor G1. Reprogramming factors OCT3/4, SOX2, CMYC and KLF4 were delivered using a non integrative methodology that involves the use of Sendai virus.

1. Resource table:

Name of stem cell line	GFM1SV.25
Institution	Departamento de Bioquímica, Instituto de Investigaciones Biomédicas "Alberto Sols", Facultad de Medicina, (UAM-CSIC) and Centro de Investigación Biomédica en Red en Enfermedades Raras (CIBERER) Madrid, Spain. Instituto de Investigación Hospital 12 de Octubre ("i + 12"), Madrid, Spain.
Person who created resource	Francisco Zurita-Díaz
Contact person and email	M. Esther Gallardo, egallardo@iib.uam.es
Date archived/stock date	August 18, 2015
Origin	Human skin cells
Type of resource	Biological reagent: induced pluripotent stem cells (iPSC) from a patient with a severe mitochondrial encephalopathy
Sub-type	Cell line
Key transcription factors	OCT3/4, SOX2, CMYC, KLF4
Authentication	Identity and purity of cell line confirmed (Fig. 1)
Link to related literature	http://www.ncbi.nlm.nih.gov/pubmed/25852744
Information in public databases	None

2. Resource details

The generation of the human iPSC line, GFM1SV.25, was carried out using non-integrative Sendai viruses containing the reprogramming factors, *OCT3/4*, *SOX2*, *CMYC*, *KLF4* (Takahashi et al., 2007). For this purpose, fibroblasts from a described patient with a severe mitochondrial encephalopathy were obtained (Brito et al., 2015). The patient's fibroblasts carried two inherited heterozygous mutations in the *GFM1* gene (c.1404delA; p.Gly469Valfs*84 and c.2011C>T; p.Arg671Cys). The presence of these mutations in the iPSC line was evaluated and confirmed by Sanger sequencing (Fig. 1A). GFM1SV.25 iPSC colonies displayed a typical ES-like colony morphology and growth behavior (Fig. 1B) and they stained positive for alkaline phosphatase activity (Fig. 1C). We confirmed the clearance of the vectors and the exogenous reprogramming factor genes by RT-PCR after eight culture passages (Fig 1D). The endogenous expression of the pluripotency associated transcription factors *OCT4*, *SOX2*, *KLF4*, *NANOG*, *CRIPTO* and *REX1* was also evaluated by RT-PCR (Fig. 1E). Immunofluorescence analysis revealed expression of transcription factors *OCT4*, *NANOG*, *SOX2* and surface markers *SSEA3*, *SSEA4*, *TRA1-60* and *TRA1-81* characteristics of pluripotent ES cells (Fig. 1F). Promoters of the pluripotency associated genes, *OCT4* and *NANOG*, heavily methylated in the original fibroblasts were almost demethylated in the GFM1SV.25 line suggesting an epigenetic reprogramming to pluripotency (Fig. 1G). The iPSC line has been adapted to feeder-free culture conditions and displays a normal karyotype (46, XX) after more than twenty culture passages (Fig. 1H). We also confirmed by DNA fingerprinting analysis that the line

* Corresponding author at: Departamento de Bioquímica, Instituto de Investigaciones Biomédicas "Alberto Sols", Facultad de Medicina (UAM-CSIC), Avda Arzobispo Morcillo s/n, 28029, Madrid, Spain.
E-mail address: egallardo@iib.uam.es (M.E. Gallardo).

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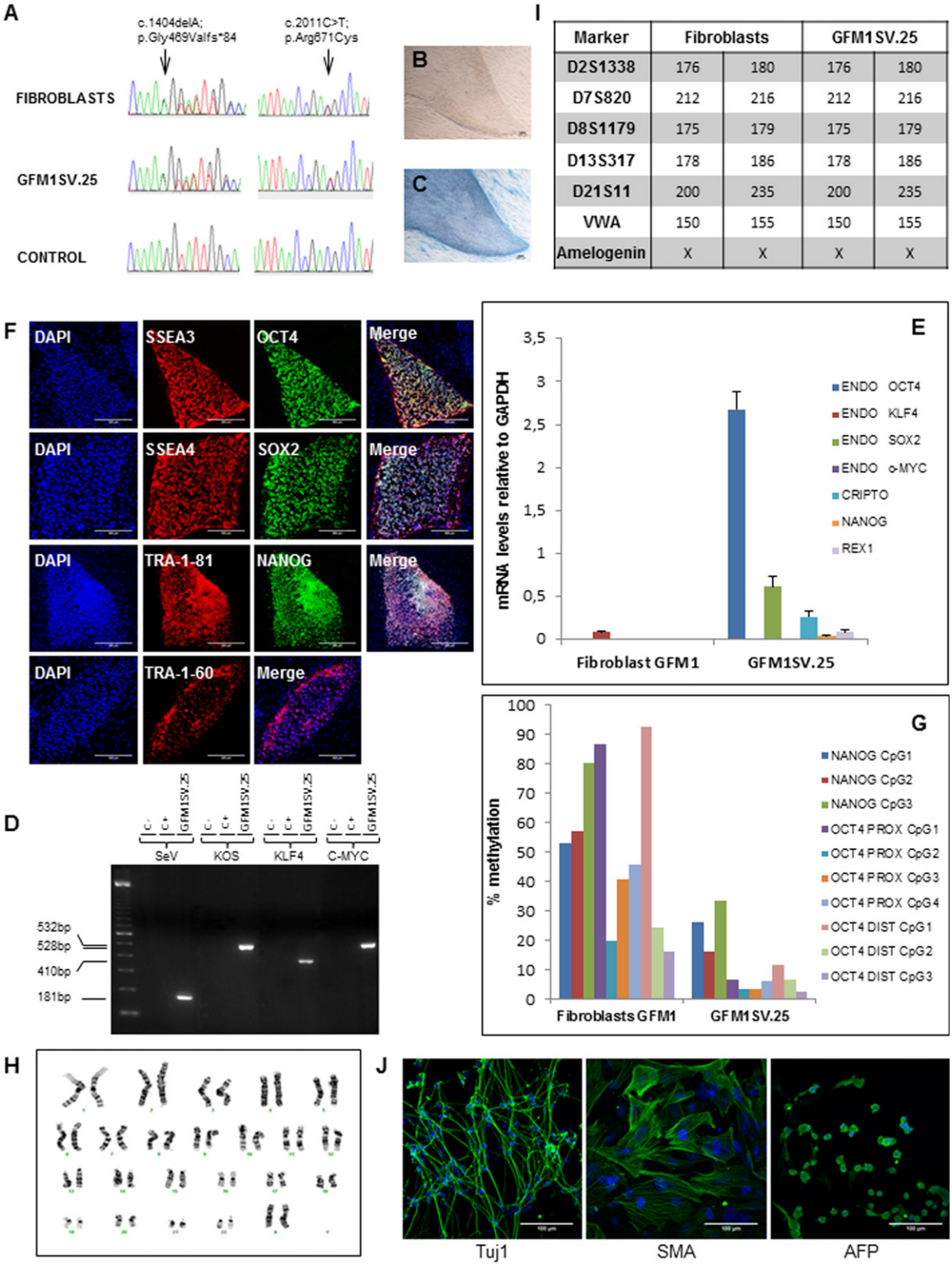

Fig. 1. Molecular and functional characterization of the GFM1SV.25 iPSC line. A. Electropherograms showing the mutations in the patient's fibroblasts and in the GFM1SV.25 line. B. Typical ES-like colony morphology of the GFM1SV.25 iPSC line. C. Positive phosphatase alkaline staining. D. RT-PCR for detecting the clearance of the vectors and the exogenous reprogramming factor genes. E. QPCR showing the expression of the pluripotency associated markers *NANOG*, *OCT4*, *SOX2*, *KLF4*, *CRIPTO* and *REX1*. F. Immunofluorescence analysis showing expression of typical pluripotent ES cell markers such as the transcription factors OCT4, NANOG, SOX2 and the surface markers SSEA3, SSEA4, TRA1-60 and TRA1-81; scale bars: 300 μm. G. Bisulfite pyrosequencing of the *OCT4* and *NANOG* promoters. The promoters of the transcription factors, *OCT4* and *NANOG* were almost demethylated in the generated iPSC line. H. Karyotype analysis. GFM1SV.25 has a normal karyotype (46, XX). I. DNA fingerprinting analysis showing that GFM1SV.25 comes from the patient's fibroblasts. 1 J. Embryoid body based in vitro differentiation assays. GFM1SV.25 differentiates into all three germ layers, demonstrated by positive AFP endoderm staining (l), positive Tuj1 ectoderm staining and positive SMA mesoderm staining.