DOI:
10.1016/j.scr.2015.12.042

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Miere, C., Hewitson, H., Wood, V., Kadeva, N., Cornwell, G., Codognotto, S., Stephenson, E., & Ilic, D. (2016). Generation of KCL021 research grade human embryonic stem cell line carrying a δF508 mutation in the CFTR gene. *Stem Cell Research*, *16*(1), 177-179. https://doi.org/10.1016/j.scr.2015.12.042

Stem Cell Research 16 (2016) 177–179

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab resource: Stem cell line

Generation of KCL021 research grade human embryonic stem cell line carrying a ΔF508 mutation in the *CFTR* gene

Cristian Miere, Heema Hewitson, Victoria Wood, Neli Kadeva, Glenda Cornwell, Stefano Codognotto, Emma Stephenson, Dusko Ilic

Stem Cell Laboratories, Division of Women's Health, Faculty of Life Sciences and Medicine, King's College London and Assisted Conception Unit, Guys' Hospital, London, United Kingdom

ARTICLE INFO

Article history:
Received 31 December 2015
Accepted 31 December 2015
Available online 6 January 2016

ABSTRACT

The KCL021 human embryonic stem cell line was derived from an embryo donated for research that carried a ΔF508 mutation affecting the *CFTR* gene encoding the cystic fibrosis transmembrane conductance regulator. The ICM was isolated using laser microsurgery and plated on γ-irradiated human foreskin fibroblasts. Both the derivation and cell line propagation were performed in an animal product-free environment. Pluripotent state and differentiation potential were confirmed by in vitro assays.

Resource table

Name of stem cell line	KCL021
Institution	King's College London, London UK
Derivation team	Neli Kadeva, Victoria Wood, Glenda Cornwell, Stefano Codognotto, Emma Stephenson
Contact person and email	Dusko Ilic, email: dusko.ilic@kcl.ac.uk
Type of resource	Biological reagent: cell line
Sub-type	Human pluripotent stem cell line
Origin	Human embryo
Key marker expression	Pluripotent stem cell markers: NANOG, OCT4, TRA-1-60, TRA-1-81, alkaline phosphatase (AP) activity
Authentication	Identity and purity of line confirmed
Link to related literature (direct URL links and full references)	1) Ilic, D., Stephenson, E., Wood, V., Jacquet, L., Stevenson, D., Petrova, A., Kadeva, N., Codognotto, S., Patel, H., Semple, M., Cornwell, G., Ogilvie, C., Braude, P., 2012. Derivation and feeder-free propagation of human embryonic stem cells under xeno-free conditions. Cytotherapy. 14 (1), 122–128.doi: 10.3109/14653249.2011.623692 http://www.ncbi.nlm.nih.gov/pubmed/22029654 2) Stephenson, E., Jacquet, L., Miere, C., Wood, V., Kadeva, N., Cornwell, G., Codognotto, S., Dajani, Y., Braude, P., Ilic, D., 2012. Derivation and propagation of human embryonic stem cell lines from frozen embryos in an animal product-free environment. Nat. Protoc. 7 (7), 1366–1381. doi: 10.1038/nprot.2012.080 http://www.ncbi.nlm.nih.gov/pubmed/22722371
Information in public databases	KCL021 is a National Institutes of Health (NIH) registered hESC line NIH Registration Number: 0219 NIH Approval Number: NIHhESC-13-0219 http://grants.nih.gov/stem_cells/registry/current.htm?id=659
Ethics	The hESC line KCL021 is derived under license from the UK Human Fertilisation and Embryology Authority (research license numbers: R0075 and R0133) and also has local ethical approval (UK National Health Service Research Ethics Committee Reference: 06/Q0702/90). Informed consent was obtained from all subjects and the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the NIH Belmont Report. No financial inducements are offered for donation.

Resource details

Consent signed	Aug. 12, 2009
Embryo thawed	Aug. 23, 2009
UK Stem Cell Bank Deposit Approval	May 23, 2011 Reference: SCSC11-14
Sex	Male 46, XY
Grade	Research
Disease status (Fig. 1)	Mutation ΔF508 affecting the *CFTR* gene encoding the cystic fibrosis transmembrane conductance regulator associated with Cystic fibrosis.
Karyotype (aCGH)	Normal
DNA fingerprint (Table 1)	Allele sizes (in bp) of 17 microsatellite markers specific for chromosomes 13, 18 and 21
Viability testing	Pass
Pluripotent markers (immunostaining) (Fig. 2)	NANOG, OCT4, TRA-1-60, TRA-1-81, AP activity
Three germ layers differentiation in vitro (immunostaining) (Fig. 3)	Endoderm: AFP (α-fetoprotein) Ectoderm: TUBB3 (tubulin, β3 class III) Mesoderm: ACTA2 (actin, α2, smooth muscle)
Sibling lines available	No

(continued on next page)

E-mail address: dusko.ilic@kcl.ac.uk (D. Ilic).

http://dx.doi.org/10.1016/j.scr.2015.12.042

Table 1
Genotyping. Microsatellite markers specific for chromosomes 13, 18, 21, X and Y were amplified. The allele sizes in bp for markers on chromosomes 13, 18, and 21 are listed in the table.

Chr	Marker	Allele 1	Allele 2
13	D13S252	299	303
	D13S305	451	454
	D13S325	285	285
	D13S628	429	454
	D13S634	401	411
	D18S386	382	382
	D18S390	372	372
	D18S391	218	218
18	D18S535	482	486
	D18S819	412	412
	D18S976	477	479
	D18S978	207	215
	D21S11	249	257
	D21S1409	212	224
21	D21S1411	308	320
	D21S1435	185	185
	D21S1437	319	339

We generated KCL021 research grade hESC line following the protocols established previously (Ilic et al., 2012; Stephenson et al., 2012). The expression of the pluripotency markers was tested after freeze/thaw cycle (Fig. 2). Differentiation potential into three germ layers was verified in vitro (Fig. 3).

Materials and methods

Consenting process

We distribute the Patient Information Sheet (PIS) and consent form to the in vitro fertilization (IVF) patients if they opted to donate to research embryos that were stored for 5 or 10 years. They mail signed consent back to us and that might be months after the PIS and consent were mailed to them. If in the meantime new versions of PIS/consent are implemented, we do not send these to the patients or ask them to re-sign; the whole process is done with the version that was given them initially. The PIS/consent documents (PGD-V.6) were created on Aug. 10, 2007. HFEA Code of Practice that was in effect at the time of document creation: Edition 7 — R.1 (http://www.hfea.gov.uk/2999.html). The donor couple signed the consent on Feb. 25, 2010. HFEA Code of Practice that was in effect at the time of donor signature: Edition 8 — R.1. HFEA Code of Practice Edition 7 — R.1 was in effect until Dec. 09, 2007 and Edition 8 — R.1 was in effect: Oct. 01, 2009–Apr. 06, 2010.

Embryo culture and micromanipulation

Embryo culture and laser-assisted dissection of inner cell mass (ICM) were carried out as previously described in details (Ilic et al., 2012; Stephenson et al., 2012). The cellular area containing the ICM was then washed and transferred to plates containing mitotically inactivated human neonatal foreskin fibroblasts (HFF).

Cell culture

ICM plated on mitotically inactivated HFF were cultured as described (Ilic et al., 2012; Stephenson et al., 2012). TE cells were removed mechanically from outgrowth (Ilic et al., 2007; Ilic et al., 2010). hESC colonies were expanded and cryopreserved at the third passage.

Viability test

Straws with the earliest frozen passage (p.2–3) are thawed and new colonies are counted three days later. These colonies are then expanded up to passage 8, at which point cells were part frozen and part subjected to standard battery of tests (pluripotency markers, in vitro and in vivo differentiation capability, genetics, sterility, mycoplasma).

Pluripotency markers

Pluripotency was assessed using two different techniques: enzymatic activity assay [alkaline phosphatase (AP) assay] and immunostaining as described (Ilic et al., 2012; Stephenson et al., 2012).

Differentiation

Spontaneous differentiation into three germ layers was assessed in vitro as described (Petrova et al., 2014; Ilic et al., 2012; Stephenson et al., 2012).

Genotyping

DNA was extracted from hESC cultures using a Chemagen DNA extraction robot according to the manufacturer's instructions. Amplification of polymorphic microsatellite markers was carried out as described (Ilic et al., 2012). Allele sizes were recorded to give a unique fingerprint of each cell line.

Array comparative genomic hybridization (aCGH)

aCGH was performed as described in details (Ilic et al., 2012).

Author disclosure statement

There are no competing financial interests in this study.

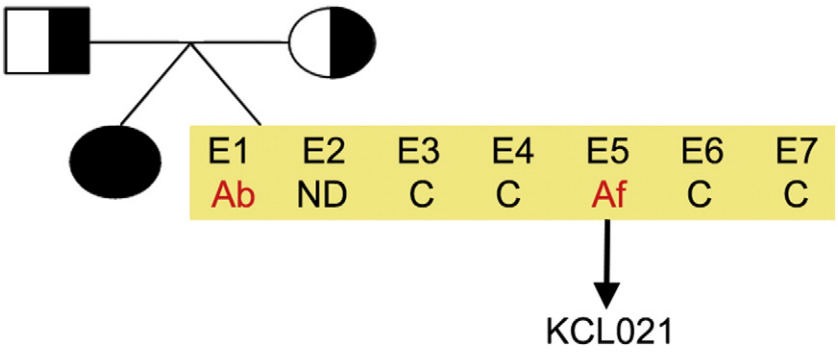

Fig. 1. Genetic pedigree tree. Couple, both carriers for ΔF508 mutation in the *CFTR* gene, with one affected child had 7 embryos in an IVF cycle. One affected embryo (E5) has been donated for research. From that embryo we derived the hESC line KCL021. Ab, abnormal; Af, affected; C, carrier.

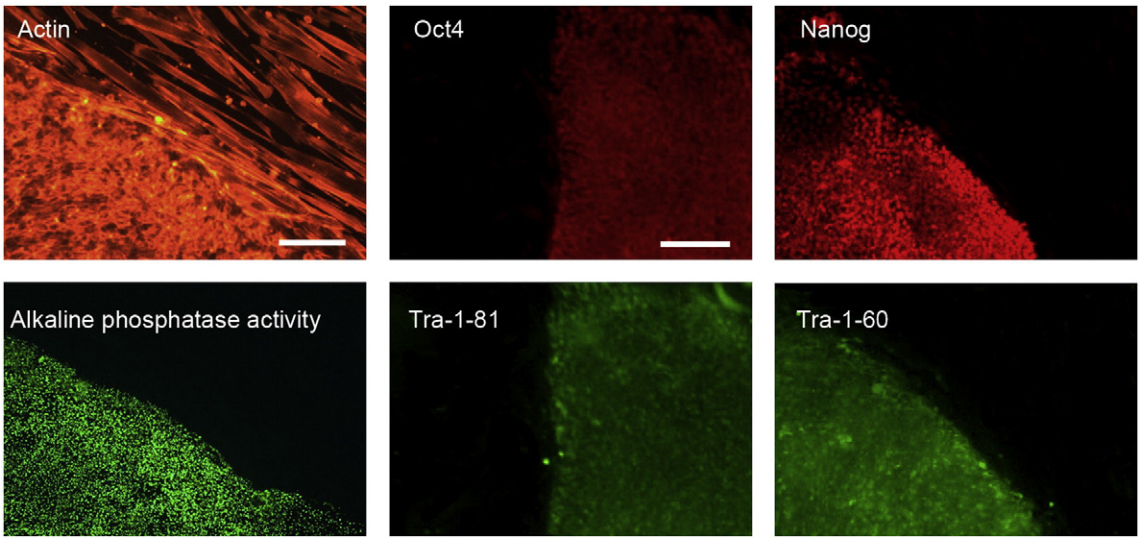

Fig. 2. Expression of pluripotency markers. Pluripotency is confirmed by immunostaining (Oct4, Nanog, TRA-1-60, TRA-1-81) and alkaline phosphatase (AP) activity assay. Actin stress fibers, visualized with rhodamine-phalloidin (red), are present in both feeders and hES cell colonies, whereas AP activity (green) is detected only in hES cells. Scale bar, 100 μm.

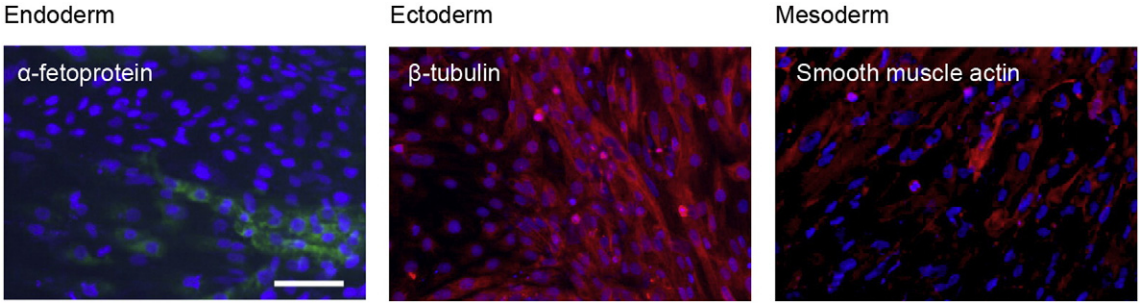

Fig. 3. Differentiation of three germ layers in vitro is confirmed by detection of markers: smooth muscle actin (red) for mesoderm, β-III tubulin (red) for ectoderm and α-fetoprotein (green) for endoderm. Nuclei are visualized with Hoechst 33342 (blue). Scale bar, 100 μm.

Acknowledgments

This work was supported by the UK Medical Research Council grants G0701172 and G0801061. We thank Dr. Yacoub Khalaf, Director of the Assisted Conception Unit of Guy's and St. Thomas' NHS Foundation Trust and his staff for supporting the research program. We are especially indebted to Prof. Peter Braude and to the patients who donated embryos.

References

Ilic, D., Genbacev, O., Krtolica, A., 2007. Derivation of hESC from intact blastocysts. Curr. Protoc. Stem Cell Biol. (Chapter 1: Unit 1 A.2).

Ilic, D., Caceres, E., Lu, S., Julian, P., Foulk, R., Krtolica, A., 2010. Effect of karyotype on successful human embryonic stem cell derivation. Stem Cells Dev. 19 (1), 39–46.

Ilic, D., Stephenson, E., Wood, V., Jacquet, L., Stevenson, D., Petrova, A., Kadeva, N., Codognotto, S., Patel, H., Semple, M., Cornwell, G., Ogilvie, C., Braude, P., 2012. Derivation and feeder-free propagation of human embryonic stem cells under xeno-free conditions. Cytotherapy 14 (1), 122–128.

Petrova, A., Celli, A., Jacquet, L., Dafou, D., Crumrine, D., Hupe, M., Arno, M., Hobbs, C., Cvoro, A., Karagiannis, P., Devito, L., Sun, R., Adame, L.C., Vaughan, R., McGrath, J.A., Mauro, T.M., Ilic, D., 2014. 3D In vitro model of a functional epidermal permeability barrier from human embryonic stem cells and induced pluripotent stem cells. Stem Cell Reports. 2 (5), 675–689.

Stephenson, E., Jacquet, L., Miere, C., Wood, V., Kadeva, N., Cornwell, G., Codognotto, S., Dajani, Y., Braude, P., Ilic, D., 2012. Derivation and propagation of human embryonic stem cell lines from frozen embryos in an animal product-free environment. Nat. Protoc. 7 (7), 1366–1381.