Lab Resource: Stem Cell Line

Generation of KCL024 research grade human embryonic stem cell line carrying a mutation in NF1 gene

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

Abstract

The KCL024 human embryonic stem cell line was derived from an embryo donated for research that carried an autosomal dominant mutation in the NF1 gene encoding neurofibromin (c.3739–3742ΔTTTG). Mutations in this gene have been linked to neurofibromatosis type 1, juvenile myelomonocytic leukemia and Watson syndrome. 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.

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Resource table

| Name of stem cell line | KCL024 |
|------------------------|--------|
| 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 |
| Date archived/stock date | Mar. 31, 2011 |
| Type of resource       | Human embryonic stem cell line |
| Sub-type               | 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) Illic, 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: 10.3109/14653249.2011.623962 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., Illic, 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

KCL024 is a National Institutes of Health (NIH) registered hESC line
NIH Registration Number: 0220
NIH Approval Number: NIHhESC-13-0220
http://grants.nih.gov/stem_cells/registry/current.htm?id=660

Ethics

The hESC line KCL024 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
Oct. 28, 2010
Embryo used
UK Stem Cell Bank Deposit Approval
Mar. 03, 2011
Sex
ND
Grade
Research
Disease status (Fig 1)
Autosomal dominant mutation in the NF1 gene encoding neurofibromin (c.3739–3742ΔTTTG)
ND
Karyotype (acGH)
DNA fingerprint
ND

(continued on next page)
Fig. 1. Genetic pedigree tree. Male donor was carrying an autosomal dominant mutation c.3739–3742 ΔTTTG in the NF1 gene. The couple undergoing IVF and prenatal genetic diagnosis had 6 embryos in this particular cycle. Embryos carrying the mutation in the NF1 gene were donated for research. We derived two hESC lines: KCL024 and KCL025.

We generated KCL024 clinical grade hESC line following protocols, established previously (Ilic et al., 2012; Stephenson et al., 2012). The expression of the pluripotency markers was tested after freeze/thaw cycle. Differentiation potential into three germ layers was verified in vitro.

| Materials and methods |
|----------------------|
| **Consenting process** |

We distribute 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 mailed 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 were created on Jul. 01, 2010. HFEA Code of Practice was in effect at the time of document creation: Edition 8 — R.2 (http://www.hfea.gov.uk/2999.html). The donor couple signed the consent on Oct. 28, 2010. HFEA Code of Practice was in effect at the time of donor signature: Edition 8 — R.2. HFEA Code of Practice Edition 8 — R.2 was in effect 07 Apr. 2010–Apr. 06, 2011.

**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 (Ilic et al., 2012; Stephenson et al., 2012; Petrova et al., 2014).

**HLA typing**

HLA-A, -B and -DRB1 typing was performed with a PCR sequence-specific oligonucleotide probe (SSOP; Luminex, Austin, TX, USA) hybridization protocol at the certified Clinical Transplantation Laboratory, Guy’s and St. Thomas’ NHS Foundation Trust and Serco Plc. (GSTS) Pathology (Guy’s Hospital, London, UK) as described (Jacquet et al., 2013).

**Author disclosure statement**

There are no competing financial interests in this study.

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**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.

Jacquet, L., Stephenson, E., Collins, R., Patel, H., Trussler, J., Al-Bedawey, R., Renwick, P., Ogilvie, C., Vaughan, R., Ilic, D., 2013. Strategy for the creation of clinical grade hESC line banks that HLA-match a target population. EMBO Mol. Med. 5 (1), 16–17.

Petrova, A., Celli, A., Jacquet, L., Dafou, D., Crumrine, D., Hupe, M., Arno, M., Hobbis, 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 Rep. 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.