Lab Resource: Stem Cell Line

Generation of KCL026 research grade human embryonic stem cell line carrying a mutation in SMN1 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 KCL026 human embryonic stem cell line was derived from an embryo donated for research that carried a mutation in the SMN1 gene encoding survival of motor neuron 1, telomeric (exons 7 and 8 deletion). Mutations in this gene are associated with spinal muscular atrophy. 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 | KCL026 |
|------------------------|--------|
| 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 | May 13, 2011 |
| Type of resource       | 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 |

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: 10.3109/14653249.2011.623692 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., 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), 1368–1381.

* Corresponding author.
  E-mail address: dusko.ilic@kcl.ac.uk (D. Illic).

Information in public databases

KCL026 is a National Institutes of Health (NIH) registered hESC line
NIH Registration Number: 0222
NIH Approval Number: NIHhESC-13-0222
http://grants.nih.gov/stem_cells/registry/current.htm?id=662

The hESC line KCL026 is derived under license from the UK Human Fertilization 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.

Consent signed Jan 12, 2011
Embryo used Apr 27, 2011
UK Stem Cell Bank Deposit Approval
Sex Male 46, XY
Grade Research
Disease status (Fig. 1) Autosomal dominant mutation in the SMN1 gene encoding neurofemin
(continued on next page)

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We generated KCL026 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 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.8) were created on Jul. 01, 2010. HFEA Code of Practice that 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 Jan. 12, 2011. HFEA Code of Practice that 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; Petrova et al., 2014).

Fig. 1. Genetic pedigree tree. The couple undergoing IVF and prenatal genetic diagnosis had 7 embryos in this particular cycle. Two embryos carrying the mutation in the SMN1 gene were donated for research. We derived hESC line KCL026 from one of the affected embryos. Af, affected; C, Carrier; N, normal.

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, 50 μm.
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).

Author disclosure statement

There are no competing financial interests in this study.

Acknowledgments

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Fig. 3. Differentiation of three germ layers in vitro is confirmed by detection of markers: smooth muscle actin (ACTA2, red) for mesoderm, β-III tubulin (TUBB3, red) for ectoderm and α-fetoprotein (AFP, red) for endoderm. Nuclei are visualized with Hoechst 33342 (blue). Scale bar, 100 μm.

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 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.