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Lab Resource: Stem Cell Line

Generation of KCL027 research grade human embryonic stem cell line carrying a mutation in the HTT gene

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A R T I C L E  I N F O

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A B S T R A C T

The KCL027 human embryonic stem cell line was derived from an embryo donated for research that carried an autosomal dominant mutation affecting one allele of the HTT gene encoding huntingtin (43 trinucleotide repeats; 21 for the normal allele). 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 and in vivo assays.

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Resource table

| Name of stem cell line | KCL027 |
|------------------------|--------|
| 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 25, 2011 |
| 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

Information in public databases

KCL027 is a National Institutes of Health (NIH) registered hESC line
NIH Registration Number: 0223
NIH Approval Number: NIHhESC-13-0223
http://grants.nih.gov/stem_cells/registry/current.htm?id=663
The hESC line KCL027 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: Jan 27, 2011
Embryo thawed: May 04, 2011
UK Stem Cell Bank Deposit Approval: Dec 01, 2011

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(continued)

| Sex                | Grade | Disease status (Fig. 1) | Karyotype (aCGH) | DNA fingerprint | HLA typing | Viability testing | Pluripotent markers (immunostaining) (Fig. 2) | Three germ layer differentiation in vitro (immunostaining) (Fig. 3) | Three germ layer differentiation in vivo (teratomas) (Fig. 4) | Targeted differentiation (Fig. 5) | Sibling lines available |
|--------------------|-------|-------------------------|-----------------|-----------------|------------|------------------|-------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|-------------------------|-------------------------|
| Male 46, XY        | Research | Mutation affecting one allele of the HTT gene encoding huntingtin (~43 CAG repeats; 21 for the normal allele) associated with Huntington’s disease (Jacquet et al., 2015) | Deletion in the chromosome 2q37.3 (242,930,539–242,948,040) × 1; known polymorphic variant. | Allele sizes (in bp) of 17 microsatellite markers specific for chromosomes 13, 18 and 21 (Jacquet et al., 2015) | HLA-A: 02,03; -B: 07,35; -C: 04,07; DRB1: 01; DQB1: 05,01; 05/01/03 | Pass | NANOG, OCT4, TRA-1-60, TRA-1-81, AP activity (Jacquet et al., 2015) | Endoderm: AFP (α-fetoprotein); Ectoderm: TUBB3 (tubulin, β3 class III); Mesoderm: ACTA2 (actin, α2, smooth muscle) (Jacquet et al., 2015) | Endoderm: AFP, GATA4. Ectoderm: TUBB3, GFAP (glial fibrillary acidic protein). Mesoderm: DES (desmin), Alcian Blue and periodic acid–Schiff (PAS)-stained cartilage (Jacquet et al., 2015) | Cardiomyocytes: TNNT2 (cardiac troponin T) immunostaining | KCL028 |

We generated KCL027 research 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 (Fig. 2; Jacquet et al., 2015). Differentiation potential into three germ layers was verified in vitro (Figs. 3 and 5; Jacquet et al., 2015) and in vivo (Fig. 4; Jacquet et al., 2015).

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 (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 at the time of donor signature: Edition 8 — R.2. 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 at the time of donor signature: Edition 8 — R.2. 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 at the time of donor signature: Edition 8 — R.2. 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 at the time of donor signature: Edition 8 — R.2. 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 at the time of donor signature: Edition 8 — R.2. 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 at the time of donor signature: Edition 8 — R.2.

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 was 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 and in vivo (Jacquet et al., 2015). Targeted differentiation in cardiomyocytes followed the protocols described earlier (Jacquet et al., 2015; Laflamme et al., 2007).

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).

HLA typing

HLA-A, -B and -DRB1 typing was performed with a PCR sequence-specific oligonucleotide probe (SSOP; Luminex, Austin, TX, USA)

![Fig. 1. Genetic pedigree tree. The couple undergoing IVF had 12 embryos in this particular cycle. Three embryos were normal, whereas nine carried the mutation in HTT and were donated for research. We derived hESC lines from two of them.](image-url)
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.

Acknowledgments

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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. Scale bar, 20 μm.

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.

Fig. 4. Differentiation of three germ layers in vivo. Teratomas were encapsulated and did not invade surrounding tissue. Sections are counterstained with hematoxylin and eosin and specific stains are brown (immunohistochemistry). Germ layer marker: DES for mesoderm, TUBB3 for ectoderm, and GATA4 for endoderm. Scale bars are 100 μm.
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