Resource table

| Name of stem cell line | KCL036 |
|------------------------|--------|
| 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 | Nov 22, 2011 |
| Type of resource       | Biological reagent: cell line |
| Origin                 | Human embryonic stem cell line |
| 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 |

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(continued)

| Information in public databases | KCL036 is a National Institutes of Health (NIH) registered hESC line |
|----------------------------------|---------------------------------------------------------------|
| NIH Registration Number          | 0241 |
| NIH Approval Number              | NIHhESC-13-0241 |
| NIH Approval Grant               | http://grants.nih.gov/stem_cells/registry/current.htm?id=668 |
| The hESC line KCL036 is derived from and approved by 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                  | Jul 21, 2011 |
|---------------------------------|-------------|
| Embryo thawed                   | Oct 23, 2011 |
| UK Stem Cell Bank               | Sep 13, 2012 |
| Deposit Approval                | Reference: SCSC12-28 |

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(continued)

| Consent signed | Jul 21, 2011 |
|----------------|-------------|
| Sex            | Male 46, XY |
| Grade          | Research    |
| Disease status | Mutation affecting one allele of the HTT gene encoding huntingtin (~38 CAG repeats; 14 for the normal allele) associated with Huntington’s disease (Ilic et al., 2015) |
| Grade          | Research    |
| Disease status | Decreased copy number at 2q37.3 (242,930,599–242,948,040) and at 3q25.1 (151,368,847–151,542,568). The imbalances are considered to be benign copy number variants. The chromosome 3 imbalance was not called by software. |
| Karyotype (aCGH)| Allele sizes (in bp) of 17 microsatellite markers specific for chromosomes 13, 18 and 21 (Jacquet et al., 2015) |
| DNA fingerprint| NaNCG, OCT4, TRA-1-60, TRA-1-81, AP activity (Jacquet et al., 2015) |
| Viability testing| Pass |
| Pluripotent markers | NANOG, OCT4, TRA-1-60, TRA-1-81, AP activity (Jacquet et al., 2015) |
| Three germ layers differentiation in vitro | Endoderm: AFP (β-fetoprotein); Ectoderm: TUBB3 (tubulin, β3 class III); Mesoderm: ACTA2 (actin, α2, smooth muscle) (Jacquet et al., 2015) |
| Three germ layer differentiation in vivo | 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) |
| Targeted differentiation | Cardiomyocytes: TNN2 (cardiac troponin T) immunostaining |
| Sibling lines available | None |

We generated KCL036 research grade hESC line following protocols established previously (Ilic et al., 2012; Stephenson et al., 2012; Jacquet et al., 2013). The expression of the pluripotency markers was established previously (Ilic et al., 2012; Stephenson et al., 2012). TE cells were re-expanded up to passage 8, at which point cells were part frozen and new colonies are counted 3 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).

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 3 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) as described (Ilic et al., 2012; Stephenson et al., 2012; Petrova et al., 2014). 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.

We also generated research grade of KCL036 line that is adapted to feeder-free conditions (Jacquet et al., 2015).

Materials and methods

Consenting process

We distribute patient information sheets (PIS) and consent forms 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. 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.9) were created on Feb. 09, 2011. 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 Jul. 21, 2011. HFEA Code of Practice that was in effect at the time of donor signature: Edition 8–R.3. HFEA Code of Practice Edition 8–R.2 was in effect: Apr. 07, 2010–Apr. 06, 2011. HFEA Code of Practice Edition 8–R.3 was in effect: Apr. 07, 2011–Oct. 01, 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).

Fig. 1. Genetic pedigree tree. The couple undergoing IVF had 16 embryos in this particular cycle. Three embryos were normal, whereas those that carried the mutation in HTT were donated for research. We derived hESC lines from two of them.
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).

**Special pathology**

The Doctors Laboratory London (UK) tested the line for HIV1, HepB, HepC, CMV, and EBV by PCR.

**Author disclosure statement**

There are no competing financial interests in this study.

**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.

**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, 25 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 AFP for endoderm. Scale bars are 100 μm.
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Fig. 5. TNNT2 (green) immunostaining on day 30 of cardiac differentiation. Nuclei are visualized with Hoechst 33342 (blue). Scale bar, 50 μm.