Lab resource: Stem cell line

**Generation of KCL031 clinical grade human embryonic stem cell line**

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**Abstract**

The KCL031 human embryonic stem cell line was derived from a normal healthy blastocyst donated for research. 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 and under current Good Manufacturing Practice (cGMP) standards. Pluripotent state and differentiation potential were confirmed by in vitro and in vivo assays.

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1. Resource table

| Name of stem cell line | KCL031 |
|------------------------|--------|
| 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 | Aug 02, 2011 |
| Type of resource       | Biological reagent: cell line |
| Sub-type Origin        | Human pluripotent stem cell line |
| Key marker expression  | Human embryo |
| Authentication          | Pluripotent stem cell markers: NANOG, OCT4, TRA-1-81, alkaline phosphatase (AP) activity |
| Identity and purity of line confirmed | 1) Jacquet, L., Stephenson, E., Collins, R., Patel, H., Trussler, J., Al-Bedaery, 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), 10–17. doi: 10.1002/emmm.201201973 http://www.ncbi.nlm.nih.gov/pubmed/23168105 |
| Informations in public databases | KCL031 is a National Institutes of Health [NIH] registered hESC line |
| NIH Registration Number: NIHhESC-14-0263 | http://grants.nih.gov/stem_cells/registry/current.htm?id=672 |

3) 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. Cytoterapy. 14 (1), 122–128. doi: 10.3109/14653249.2011.623692 http://www.ncbi.nlm.nih.gov/pubmed/22029654

4) 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

Ethics

KCL031 is a National Institutes of Health (NIH) registered hESC line

NIH Registration Number: NIHhESC-14-0263

http://grants.nih.gov/stem_cells/registry/current.htm?id=672

The hESC line KCL031 is derived under license from the UK Human Fertilisation and Embryology Authority (research licence 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.

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2. Resource details

| Consent signed | Nov 26, 2008 |
|---|---|
| Embryo thawed | Jun 29, 2011 |
| UK Stem Cell Bank Deposit Approval | Reference: SCSC12-37 |
| Sex | Male 46, XY |
| Grade | Clinical |
| Disease status | Healthy/Unaffected |
| Karyotype (aCGH) | 50 kb deletion at 7q22.3 (105,465,968–105,516,305), Loss at 8q24.23 (136,718,037–136,837,768) (Canham et al., 2015) |
| SNP Array | (Canham et al., 2015) |
| DNA fingerprint | Allele sizes (in bp) of 16 microsatellite markers specific for chromosomes 13, 18 and 21 (Jacquet et al., 2013) |
| HLA typing | HLA-A 02, 24; B 51, 52; Bw 4; C 12, 14; DRB1 11, 15; DRB3 02; DRB5 01; DQB1 03, 06 (Jacquet et al., 2013; Canham et al., 2015) |
| Viability testing | Pass |
| Mycoplasma | Negative |
| Sterility | Pass |
| Pluripotent markers (immunostaining) | NANOG, OCT4, TRA-1-60, TRA-1-81, AP activity (Fig. 1) |
| Three germ layer differentiation in vitro (immunostaining) (Fig. 2) | Endoderm: AFP (α-fetoprotein) Ectoderm: TUBB3 (tubulin, beta 3 class III) Mesoderm: ACTA2 (actin, alpha 2, smooth muscle) Endoderm: AFP, GATA4 |
| Three germ layer differentiation in vivo (teratomas) (Fig. 3) | Ectoderm: TUBB3, GFAP (glial fibrillary acidic protein) Mesoderm: DES (desmin), Alcian Blue and periodic acid–Schiff (PAS)-stained cartilage Cardiomyocytes: TNNT2 (cardiac troponin T) immunostaining |
| Targeted differentiation (Fig. 4) | |
| Sibling lines available | No |

We generated KCL031 clinical grade hESC line following protocols, established previously (Ilic et al., 2012; Stephenson et al., 2012), and now adapted to cGMP conditions. The expression of the pluripotency markers was tested after freeze/thaw cycle (Fig. 1). Differentiation potential into three germ layers was verified in vitro (Fig. 2) and in vivo (Fig. 3), as well as targeted differentiation into cardiac myocytes (Fig. 4).

Molecular karyotyping using array comparative genomic hybridization aCGH identified deletion at 7q22.3 (105,465,968–105,516,305). Whole-genome single nucleotide polymorphism (SNP) array analysis detected loss at 8q24.23 (136,718,037–136,837,768) (Canham et al., 2015). The gain contains no genes and it has been also reported previously to occur in healthy individuals from worldwide population (Macdonald et al., 2014). Estimated frequency in the human population is 3.85% (Canham et al., 2015).

Donors were tested negative for Human Immunodeficiency Virus 1 (HIV1), Hepatitis B (HepB, HBc) and C Virus (HepC, HCV). We did not retest the line.

We also generated research grade of KCL031 line that is adapted to feeder-free conditions.

3. Materials and methods

3.1. 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 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 (FRO-V.5) 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 Nov. 26, 2008. HFEA Code of Practice that was in effect at the time of donor signature: Edition 7 — R.4. HFEA Code of Practice Edition 7 — R.1 was in effect until Dec. 09, 2007, whereas 7 — R.4 was in effect: Oct. 02, 2008–Sep. 30, 2009.

3.2. 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).

3.3. 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.

3.4. 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).

3.5. Pluripotency

Pluripotency in vitro 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).

3.6. Differentiation

Spontaneous differentiation into three germ layers was assessed in vitro and in vivo as described (Stephenson et al., 2012; Petrova et al., 2014). Targeted differentiation in cardiomyocytes followed the protocols described earlier (Laflamme et al., 2007; Jacquet et al., 2015).

Fig. 2. 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 (red) for endoderm. Nuclei are visualized with Hoechst 33,342 (blue). Scale bar, 50 μm.

Fig. 3. 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) or light blue (Alcian blue). Germ layer markers: Alcian blue–PAS–stained cartilage and DES for mesoderm, TUBB3 and GFAP for ectoderm, GATA4 and AFP for endoderm. Positive immunostaining for complex IV type II marker confirms the human origin of the tumor (adjacent section of the one stained for desmin). Scale bars are 100 μm.
3.7. 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.

3.8. Array comparative genomic hybridization (aCGH)

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

3.9. Whole-genome single nucleotide polymorphism (SNP) array

SNP array was performed as described in details (Canham et al., 2015).

3.10. 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). HLA typing was also performed independently by other group (Canham et al., 2015).

4. Author disclosure statement

There are no competing financial interests in this study.

Acknowledgments

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Fig. 4. TNNT2 (green) immunostaining on day 30 of cardiac differentiation. Nuclei are visualized with Hoechst 33,342 (blue). Scale bar, 10 μm.