Lab Resource: Stem Cell Line

Generation of KCL033 clinical grade human embryonic stem cell line

Liani Devito, Anastasia Petrova, 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

A R T I C L E   I N F O

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A B S T R A C T

The KCL033 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 assays. The line was also validated for sterility and specific and non-specific human pathogens.

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Resource table

| Name of stem cell line | KCL033 |
|------------------------|--------|
| 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 17, 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 | 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/23161805
2) Canham, A., Van Deusen, A., Brison, D.R., De Sousa, P., Downie, J., Devito, L., Hewitt, Z.A., Ilic, D., Kimber, S.J., Moore, H.D., Murray, H., Kunath, T., 2015. The molecular karyotype of 25 clinical-grade human embryonic stem cells lines. Sci. Rep. 5, 17258. doi: 10.1038/srep17258 http://www.ncbi.nlm.nih.gov/pubmed/26609762
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: 10.3109/14653249.2011.622092 http://www.ncbi.nlm.nih.gov/pubmed/22029854
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5) Devito, L., Petrova, A., Miere, C., Codognotto S., Blakely, N., Lovatt, A., Ogilvie, C., Khalaf, Y., Ilic, D., 2014. Cost-effective master cell bank validation of multiple clinical-grade human pluripotent stem cell lines from a single donor. Stem Cells Transl. Med. 3(10), 1116–1124. doi: 10.5966/sctm.2014-0015 http://www.ncbi.nlm.nih.gov/pubmed/25122650 |
| Information in public databases | KCL033 is a National Institutes of Health (NIH) registered hESC line
NIH Registration Number: NIHhESC-14-0267 http://grants.nih.gov/stem_cells/registry/current.htm?id=653 |
| Ethics | The hESC line KCL033 is derived under license from the UK Human Fertilisation and Embryology Authority (research |

* Corresponding author.
We generated KCL033 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).

Molecular karyotyping identified a novel 2.4 Mb gain on chromosome 5p14.3 and a gain on chromosome 12p11.21, which was also found in KCL040.

The gain on chromosome 5p14.3 containing a single gene, CDH18 (Cadherin-18), was also present in one of two sibling cell lines, KCL032, but not in KCL034, a third sibling. A duplication of this size has not been reported to date, but its presence in two sibling cell lines strongly suggests that it was inherited from one of the parents rather than by acquisition during hESC derivation and culture (Canham et al., 2015). The 2498.8 bp gain starts at bp 19086546 and ends at bp 21585311 as referred to Human Genome Build 38.

The gain on chromosome 12p11.21 contains no genes and it has not been reported to date, but its presence in two sibling cell lines, KCL032, but not in KCL034, a third sibling. A duplication of this size has been also reported in at least 14 submissions at Database of Genomic Variants (DGV; http://dgv.tcag.ca), which has collected structural variations in more than 14,000 healthy individuals from worldwide population (MacDonald et al., 2014). Estimated frequency in the human population is 4.70% (Canham et al., 2015).

Validation for sterility and specific and non-specific human pathogens (Devito et al., 2014) confirmed that the cells in the Master Bank were sterile, mycoplasma-free, and negative for Treponema pallidum, chlamydia, Neisseria gonorrhoeae, human immunodeficiency virus-1 and 2 (HIV-1 and -2), human T-lymphotropic virus types-1 and 2 (HTLV-1 and -2), hepatitis A, B and C (HAV, HBV and HCV), human herpes simplex virus HHV-4 (Epstein–Barr virus, EBV), -6, -7, and -8, human cytomegalovirus (HCMV), human parvovirus B19, SV40, JCV, BKV, enterovirus, HAV, HCV, nonspecific viral and other adventitious contaminants.

We also generated research grade of KCL033 line that is adapted to feeder-free conditions.

### Resource details

| Consent signed | May 26, 2009 |
|----------------|-------------|
| Embryo thawed  | Jul 11, 2011|
| UK stem cell bank deposit approval | Mar 08, 2012 |
| Sex            | Female 46, XX |
| Grade          | Clinical |
| Disease status | Healthy/Unaffected |
| Karyotype (aCGH) | No copy number changes detected. |
| SNP array      | Gain in regions 5p14.3 and 12p11.21 (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 11,29; B 44,51; Bw 4; C 04,16; DRB1 04,07; DRB4 01; DQB1 02,03 (Jacquet et al., 2013; Canham et al., 2015) |
| Viability testing | Pass |
| Mycoplasma     | Negative |
| Sterility      | Pass |
| Pluripotent markers (immunostaining) (Fig. 1) | NANOG, OCT4, TRA-1-60, TRA-1-81 |
| Three germ layers differentiation in vitro (immunostaining) (Fig. 2) | Endoderm: AFP; Ectoderm: TUBB3 (tubulin, beta 3 class III); Mesoderm: ACTA2 (actin, alpha 2, smooth muscle) |
| Sibling lines available | KCL032, KCL034 |

Embryo culture and micromanipulation

Embryo culture and laser-assisted dissection of inner cell mass (ICM) were carried out as previously described in detail (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 the outgrowth (Ilic et al., 2007; Ilic et al., 2010). hES colonies were expanded and cryopreserved at the third passage.

![Fig. 1. Expression of pluripotency markers.](image)
**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 with immunostaining for pluripotency markers as described (Ilic et al., 2012; Stephenson et al., 2012).

**Differentiation**

Spontaneous differentiation into three germ layers was assessed in vitro and in vivo as described (Petrova et al., 2014; Stephenson et al., 2012).

**Genotyping**

DNA was extracted from hES cell 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 detail (Ilic et al., 2012).

**Whole-genome single nucleotide polymorphism (SNP) array**

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

**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).

**Validation for sterility and specific and non-specific human pathogens**

Validation for sterility and specific and non-specific human pathogens was performed as described (Devito et al., 2014). All validation studies were conducted by SGS Vitrology (Glasgow, U.K., http://www.sgs.com), in compliance with the principles of GMP as set out in Directive 2003/94/EC for medicinal products for human use (Directive 2003/94/EC, 2003) and 91/412/EEC for veterinary medicinal products (Directive 91/412/EEC, 1991).

Sterility testing was performed in accordance with the current requirements of the European Pharmacopoeia, Section 2.6.1 Sterility, U.S. Pharmacopeia, 71. Sterility Tests, and International Conference on Harmonisation Topic Q5D guidelines.

Mycoplasma testing was performed in accordance with the current requirements of the European Pharmacopoeia, Section 2.6.7, Mycoplasmas.

All PCR-based assays used were compliant with the current edition of the European Pharmacopoeia, 2.6.21, Nucleic Acid Amplification Techniques.

**Author disclosure statement**

There are no competing financial interests in this study.

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