Lab resource: Stem cell line

Generation of KCL037 clinical grade human embryonic stem cell line

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ABSTRACT

The KCL037 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.

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1. Resource table

| Name of stem cell line | KCL037 |
|------------------------|--------|
| 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. 21, 2011 |
| Type of resource       | Biological reagent: 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) Jacquet, L., Stephenson, E., Collins, R., Patel, H., Trussier, 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. http://dx.doi.org/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 cell lines. Sci. Rep. 5, 17258. http://dx.doi.org/10.1038/srep17258

3) Ilic, D., Stephenson, E., Wood, V., Jacquet, L., Stevenson, D., Petrowa, 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. http://dx.doi.org/10.1016/j.scr.2015.12.023 1873-5061/© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). |

2. Resource details

| Consent signed | Sep. 22, 2009 |
| Embryo thawed | Oct. 18, 2011 |
| UK Stem Cell Bank Deposit Approval | Reference: |
| Sex | Male 46, XY |

* Corresponding author. E-mail address: dusko.ilic@kcl.ac.uk (D. Ilic).
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 (PFS-R1.4) were created on Dec. 18, 2008. HFEA Code of Practice that was in effect at the time of document creation: Edition 6 – R.4 (http://www.hfea.gov.uk/2999.html). The donor couple signed the consent on Sep. 22, 2009. HFEA Code of Practice that was in effect at the time of donor signature: Edition 6 – R.4. HFEA Code of Practice Edition 6 – R.4 was in effect: 02 Oct. 2008 – 30 Sep. 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). hES 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).

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**Grade Clinical**

**Disease status Healthy/Unaffected**

**Karyotype (aCGH)** Decreased copy number at 2q37.3 and 3q25.1. Both are considered to be benign copy number variants.

**SNP Array** Gain on chromosome 18q23 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 02,03; B 35,40; Bw 06; C 03,04; DRB1 01,13; DRB3 03; DQB1 05,06 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**

**Three germ layers differentiation in vitro (immunostaining)**

- **Endoderm:** AFP
- **Ectoderm:** TUBB3 (tubulin, beta 3 class III)
- **Mesoderm:** ACTA2 (actin, alpha 2, smooth muscle)

**Sibling lines available No**

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We generated KCL037 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 decreased copy number at 2q37.3 (242,930,599–242,948,040) and 3q25.1 (151,368,847–151,542,568). The imbalances identified are considered to be benign copy number variants. The chromosome 3 imbalance was not “called” by software.

Whole-genome single nucleotide polymorphism (SNP) array analysis revealed a 542 kb gain on chromosome 18q23 in KCL037 containing two coding genes, **SALL3** and **ATP9B** (Canham et al., 2015). A smaller duplication (Cnvsv57794) covering the same two genes has been reported previously (Cooper et al., 2011).

We also generated research grade of KCL037 line that is adapted to feeder-free conditions.
3.5. 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).

3.6. Differentiation

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

3.7. 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.

3.8. Array comparative genomic hybridization (aCGH)

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

3.9. SNP array

SNP array was performed as described in detail (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).

Author disclosure statement

There are no competing financial interests in this study.

Acknowledgments

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References

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.

Cooper, G.M., Coe, B.P., Girirajan, S., Rosenfeld, J.A., Vu, T.H., Baker, C., Williams, C., Stalker, H., Hamid, R., Hannig, V., Abdel-Hamid, H., Bader, P., McCracken, E., Niyazov, D., Leppig, K., Thiése, H., Hummel, M., Alexander, N., Gorski, J., Kussmann, J., Shashi, V., Johnson, K., Rehder, C., Ballif, B.C., Shaffer, L.G., Eichler, E.E., 2011. A copy number variation morbidity map of developmental delay. Nat. Genet. 43 (9), 838–846.

Ilic, D., Genbacev, O., Krtolica, A., 2007. Derivation of hESC from intact blastocysts. Curr. Protoc. Stem Cell Biol. (Chapter 1: Unit 1A.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.

Jacquet, L., Stephenson, E., Collins, R., Patel, H., Trussler, J., Al-Bedair, 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.

Petrova, A., Celli, A., Jacquet, L., Dafou, D., Crumrine, D., Hupe, M., Arno, M., Ansell, J., Cтворo, 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.

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.