Lab resource: Stem cell line

Generation of KCL029 research grade human embryonic stem cell line carrying a mutation in WAS gene

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A B S T R A C T

The KCL029 human embryonic stem cell line was derived from an embryo donated for research that carried a c.814 T > C mutation in the WAS gene, which is linked to the Wiskott-Aldrich syndrome, a rare, inherited, X-linked, recessive disease characterized by immune dysregulation and microthrombocytopenia. The line is also carrier for a mutation p.N1152H in the gene encoding the cystic fibrosis transmembrane conductance regulator CFTR. 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 assays.

1. Resource table

| Name of stem cell line | KCL029 |
|------------------------|--------|
| Institution            | King’s College London, London UK |
| Derivation team        | Neli Kadeva, Victoria Wood, Glenda Cornwell, Stefano Codognotto, Emma Stephenson |
| Contact person and email | Dusko Illic, email: dusko.ilic@kcl.ac.uk |
| 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 |
|                       | 1) Illic, 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 |
|                       | 2) Stephenson, E., Jacquet, L., Miere, C., Wood, V., Kadeva, N., Cornwell, G., Codognotto, S., Dajani, Y., Braude, P., Illic, 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 |

KCL029 is a National Institutes of Health (NIH) registered hESC line

Information in public databases
- NIH Registration Number: 0225
- NIH Approval Number: NIHhESC-13-0225
- http://grants.nih.gov/stem_cells/registry/current.htm?id=658

Ethics
- The hESC line KCL029 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.

2. Resource details

Consent signed
- Aug 12, 2009

Embryo thawed
- Aug 23, 2009

UK Stem Cell Bank
- Dec 01, 2011

Deposit Approval
- Reference: SCSC11-46

Sex
- Male 46, XY

Grade
- Research

Disease status (Fig. 1)
- c.814 T > C mutation in the WAS gene and carrier for a mutation p.N1152H in the gene CFTR

Karyotype (aCGH)
- Duplication of approximately 0.23 Mb from the long arm of chromosome 3: 3q29(197,574,292–197,803,820) x 3
We generated KCL029 clinical 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). Differentiation potential into three germ layers was verified in vitro (Fig. 3).

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 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. 20, 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 Apr. 07, 2010 – Apr. 06, 2011.

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 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; Ilic et al., 2012; Stephenson et al., 2012).

Table 1

| Chr | Marker | Allele 1 | Allele 2 |
|-----|--------|----------|----------|
| 13  | D13S252| 294      | 298      |
|     | D13S305| 447      | 455      |
|     | D13S325| 284      | 293      |
|     | D13S628| 457      | 457      |
|     | D13S634| 405      | 415      |
| 18  | D18S386| 352      | 375      |
|     | D18S390| 360      | 372      |
|     | D18S391| 217      | 225      |
|     | D18S535| 482      | 482      |
|     | D18S819| 400      | 408      |
|     | D18S976| 476      | 480      |
|     | D18S978| 207      | 211      |
| 21  | D21S11 | 248      | 248      |
|     | D21S1409| 212      | 212      |
|     | D21S1411| 303      | 308      |
|     | D21S1435| 184      | 188      |
|     | D21S1437| 311      | 315      |

Table 1 (continued)

| DNA fingerprint (Table 1) | Allele sizes (in bp) of 17 microsatellite markers specific for chromosomes 13, 18, and 21 |
|---------------------------|-----------------------------------------------------------------------------------------------|
| Viability testing         | Pass                                                                                           |
| Pluripotent markers       | Nanog, Oct4, TRA-1-60, TRA-1-81, AP activity                                                   |
| (immunostaining)          |                                                                                               |
| (Fig. 2)                  |                                                                                               |
| Three germ layers         | Endoderm: AFP (α-fetoprotein)                                                                   |
| differentiation in vitro  | Ectoderm: TUBB3 (tubulin, β3 class III)                                                         |
| (immunostaining)          | Mesoderm: ACTA2 (actin, α2, smooth muscle)                                                     |
| (Fig. 3)                  |                                                                                               |
| Sibling lines available   | No                                                                                             |

Genotyping. Microsatellite markers specific for chromosomes 13, 18, 21, X and Y were amplified. The allele sizes in bp for markers on chromosomes 13, 18, and 21 are listed in the table.
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).

Author disclosure statement

There are no competing financial interests in this study.

Acknowledgments

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