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Lab Resource: Stem Cell Line

Derivation of the human embryonic stem cell line RCM1

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ABSTRACT

The human embryonic stem cell line RCM-1 was derived from a failed to fertilise egg undergoing parthenogenetic stimulation. The cell line shows normal pluripotency marker expression and differentiation to three germ layers *in vitro* and *in vivo*. It has a normal 46XX female karyotype and microsatellite PCR identity, HLA and blood group typing data is available.

Resource table

Name of stem cell construct	RCM1
Institution	Roslin Cells Ltd. St Mary's Hospital, Manchester
Person who created resource	B. Tye, J. Gardner, C. Pope, S. Sneddon, K. Bruce, P. Dand, G. Russell, D.M. Collins, A. Greenshields, K. McDonald, H. Bradburn
Contact person and email	Paul.desousa@roslincells.com; Paul.desousa@ed.ac.uk; Janet.downie@roslincells.com Aidan.courtney@roslincells.com daniel.brison@manchester.ac.uk
Date archived/stock date	21 December 2006 (passage 10 on feeders) 07 February 2011 (seed bank at passage 24)
Type of resource	Biological reagent: Cell line
Sub-type	hESC, research grade
Origin	Blastocyst with ICM and trophoblast originating from clinically failed egg receiving parthenogenetic stimulus. Confirmed as biparental by whole genome SNIP analysis.
Key transcription factors	Oct4 (confirmed by flow cytometry and immunocytochemistry)
Authentication	See Quality Control Test Summary, Table 1
Link to related literature (direct URL links and full references)	De Sousa PA*, Gardner J, Sneddon S, Pells S, Jorgenson Tye B, Dand P, Collins DM, Steward K, Shaw L, Przyborski S, Cooke M, McLauglin KJ, Kimber SJ, Lieberman BA, Wilmut I, Brison D. (2009) Clinically failed eggs as a source of normal human embryo stem cells. Stem Cell Research 2, 188–197. http://www.sciencedirect.com/science/article/pii/S1873506109000026
Information in public databases	http://www.nibsc.org/science_and_research/advanced_therapies/uk_stem_cell_bank/cell_lines.aspx
Ethics	Informed consent obtained. Approved by participating institutional ethics advisory boards and Central Manchester Local Research Ethics Committee reference number 03/CM/684. Conducted under the UK Human Fertilisation and Embryology Authority licence no R0136.

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Resource details.

RCM1 was derived from a failed to fertilise egg subjected to parthenogenic activation. The cell line was derived by whole embryo outgrowth on mitotically inactivated human fibroblast (HDF) feeder cells using HDF conditioned medium and expanded under feeder free conditions.

RCM1 was shown to be pluripotent by expression of Oct 4 using immunocytochemistry (Table 1, Fig. 1). By flow cytometric analysis, the expression of pluripotency makers Oct4, Tra-1-60 and SSEA-4 was 97%, 98% and 99%, respectively, whereas low expression of the differentiation marker SSEA-1 (6%) was observed (Fig. 2).

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Table 1
Summary of quality control testing and results for RCM-1.

Classification	Test	Purpose	Result
Donor screening	HIV 1 + 2 Hepatitis B Hepatitis C	Donor screening for adventitious agents	Negative
Identity	Microsatellite PCR (mPCR)	DNA Profiling to give cell line its signature, gender/species	Performed
Phenotype	Immunocytochemisty	To assess levels of staining for the pluripotency markers Oct4 and Nanog	Expression of Oct4 and Nanog
	Flow cytometry	Assess antigen levels & cell surface markers commonly associated with hESC	Oct3/4: 97% Tra 1-60: 98% SSEA-4: 99% SSEA-1: 6%
Genotype	Blood group genotyping (DNA analysis)	To establish blood group of the line	AA
	Karyology (G-banding)	Confirmation of normal ploidy by G-banding	46XX
	HLA tissue typing	To establish full HLA Type I and Type II genotypes of the line	HLA typed Class I and Class II
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-qPCR	Negative
Morphology	Photography	To capture a visual record of the line	Normal
Differentiation potential	Embryoid body formation	To show differentiation to three germ layers	Expression of muscle actin, β-tubulin and α-feto protein
	Teratoma formation	To show differentiation to three germ layers	Teratoma formation observed, three germ layers detected

Differentiation to the three germ layers, endoderm, ectoderm and mesoderm, was demonstrated using embryoid body formation and teratoma formation (Fig. 1).

A microsatellite PCR profile has been obtained for the cell line, and HLA Class I and Class II typing is available (Table 1). Blood group genotyping gave the blood group AA (Table 1). The cell line was analysed for genome stability by G-banding and GCH (Fig. 3), as well as a SNP array screen to determine whether RCM1 was derived from a parthenogenetic or fertilized embryo. This showed a normal, 46XX female genotype with no genetic deficiencies that had originated from a fertilized egg.

The cell line is free from mycoplasma contamination as determined by RC-qPCR.

Materials and methods

Ethics

Derivation of hESC from surplus to requirement and failed to fertilise/develop embryos was approved by The Scotland A Research Ethics Committee and local ethics board at participating fertility clinics and conducted under licence no R0136 from the UK HFEA with informed donor consent.

Cell culture

Activation and derivation of RCM1 is described in De Sousa et al. (2009), Stem Cell Research 2, 188–197. The established cell line was expanded and banked using CellStart matrix and Stempro hESC Serum Free Medium (ThermoFisher Scientific (Life Technologies), Paisley, UK). Passaging was performed mechanically using an EZ passage tool (ThermoFisher Scientific).

Mycoplasma

Mycoplasma detection was performed using Applied Biosystems PrepSEQ™ Mycoplasma Nucleic Acid Extraction Kit and MicroSEQ™ Mycoplasma Real-Time PCR Detection Kit (ThermoFisher Scientific (Applied Biosystems)) according to the manufacturer's instruction.

Flow cytometry

Pluripotency was determined using the Human and Mouse Pluripotent Stem Cell Analysis kit (BD, Oxford, UK). Oct3/4 and SSEA-4 were included as pluripotency markers, and SSEA-1 as a differentiation marker. FITC conjugated Tra-1-60 (BD) was used as an additional pluripotency marker. Fixed and permeabilised cells were analysed using the Guava easyCyte flow cytometer (Millipore, Watford, UK).

Immunocytochemistry

hESC were fixed in 4% paraformaldehyde (ThermoFisher Scientific (Alfa Aesar)), permeabilised using 100% ethanol (ThermoFisher Scientific) and stained with AFP (1:500; Sigma Aldrich, Dorset, UK), β-tubulin III (1:1000; Sigma), muscle-specific actin (1:50; DAKO, Glostrup, Denmark), Oct4 (1:200; Santa Cruz Biotechnology, Texas, USA), Nanog (1:20; R&D Systems, Abingdon, UK) and secondary antibodies anti-mouse IgG-FITC (1:200; Sigma), anti mouse IgG — AlexaFluor 488, anti-goat IgG — AlexaFluor 488 and anti-goat IgG — AlexaFluor-594 (1:200 donkey polyclonal AlexaFluor-594) (all 1:200; ThermoFisher Scientific). Alkaline Phosphatase staining was as per manufacturer's recommended protocol (Vector labs, Peterborough, UK). Images were acquired using a Zeiss S100 Axiovert fluorescence microscope or Nikon eC1 confocal microscope.

In vitro differentiation

hESC cells were pre-treated for 1 h with 10 μM ROCK inhibitor in Stempro hESC SFM (ThermoFisher Scientific) and embryoid bodies EBs generated in ultra low attachment plates (Corning) for 7 days before being transferred into EB medium (20% FBS (GE Healthcare (PAA), Buckinghamshire, UK), 80% KO-DMEM 1 mM L-glutamine, 0.1 mM β-mercaptoethanol, 1% nonessential amino acids (all ThermoFisher Scientific)), on glass slide tissue culture chambers