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Lab Resource

Derivation of HVR1, HVR2 and HVR3 human embryonic stem cell lines from IVF embryos after preimplantation genetic diagnosis (PGD) for monogenic disorder

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ABSTRACT

From 106 human blastocyts donate for research after in vitro fertilization (IVF) and preimplantation genetic diagnosis (PGD) for monogenetic disorder, 3 human embryonic stem cells (hESCs) HVR1, HVR2 and HVR3 were successfully derived. HVR1 was assumed to be genetically normal, HVR2 carrying Becker muscular dystrophy and HVR3 Hemophilia B. Despite the translocation t(9;15)(q34.3;q14) detected in HVR2, all the 3 cell lines were characterised in vitro and in vivo as normal hESCs lines and were registered in the Spanish Stem Cell Bank.

Resource table

Name of stem cell lines	HVR1, HVR2, HVR3
Institution	Andalusian Center for Molecular Biology and Regenerative Medicine – Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER) Unidad de Gestión Clínica de Genética, Reproducción y Medicina Fetal (UGC) – University Hospital Virgen del Rocío
Persons who created resource	Aguilera Y, and Lozano-Arana MD
Contact person and email	Hmadcha A, karim.hmadcha@cabimer.es Antiñolo G, guillermo.antinolo.sspa@juntadeandalucia.es Soria B, bernat.soria@cabimer.es
Date archived/stock date	HVR1: March 2009 HVR2: December 2009 HVR3: December 2010
Origin	Human embryos donated for research from couples undergoing IVF treatment and from couple included in our PGD program.
Type of resource	Derived human embryonic stem cell lines
Sub-type	Cell lines
Key transcription factors	Oct4, Sox2, Nanog, TRA-1-60 and SSEA4
Authentication	Identity and purity of cell line confirmed (Fig. 1)
Link to related literature (direct URL links and full references)	Not available
Information in public databases	http://www.isciii.es/ISCIII/es/contenidos/fd-el-instituto/fd-organizacion/fd-estructura-directiva/fd-subdireccion-general-investigacion-terapia-celular-medicina-regenerativa/fd-centros-unidades/fd-banco-nacional-lineas-celulares/fd-lineas-celulares-disponibles/lineas-de-celulas-hES.shtml
Ethical approval	Human embryos that had been donated for research after IVF and PGD program were used for this study with the informed consent of the couples and the approval of the ethical committee at the University Hospital Virgen del Rocio.

Resource details

The hESC lines HVR1, HVR2 and HVR3 were derived within a research project entitled "Derivation of hESC lines of pre-embryos affected

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by genetic diseases obtained after PGD", after approval from the Regional Commission Clinical Research Ethics Board of the Health Ministry of Junta de Andalucía (Inf. No. 4/07).

A total of 9 embryos from in vitro fertilization (IVF) and 97 embryos from preimplantation genetic diagnosis (PGD) were donated for research in accordance with the legal requirements of the country of origin by donors included in PGD program at Unidad de Gestión Clínica de Genética, Reproducción y Medicina Fetal (UGC) from University Hospital Virgen del Rocío. The donors gave written informed consent (Cortes JL et al., 2007 and Fernández et al., 2014).

We were able to derive 3 embryonic stem cell (hESC) lines (HVR1, HVR2 and HVR3). The HVR1 was derived from human embryo donated for research from a healthy couple undergoing IVF treatment; HVR2 and HVR3 from human embryo donated for research from a couple included in our PGD program for Becker muscular dystrophy for Hemophilia A respectively.

Human embryos were thawed using Thaw Kit 1TM de Vitrolife® and cultured using G2 medium (GIII series, Vitrolife®), inner cell mass (ICM) was mechanically isolated under stereomicroscope using 2 insulin syringe (25G) at "hatching blastocyst" stage (Ström et al., 2007) and plated onto mitomycin-C inactivated human newborn foreskin fibroblasts (hFFs), the resulting colonies displayed the typical morphology of hESCs (Fig. 1A) and are positive to alkaline phosphatase staining (Fig. 1B).

The analysis of pluripotent markers was evaluated by RT–PCR, immunofluorescence and flow cytometry. Undifferentiated HVR1, HVR2

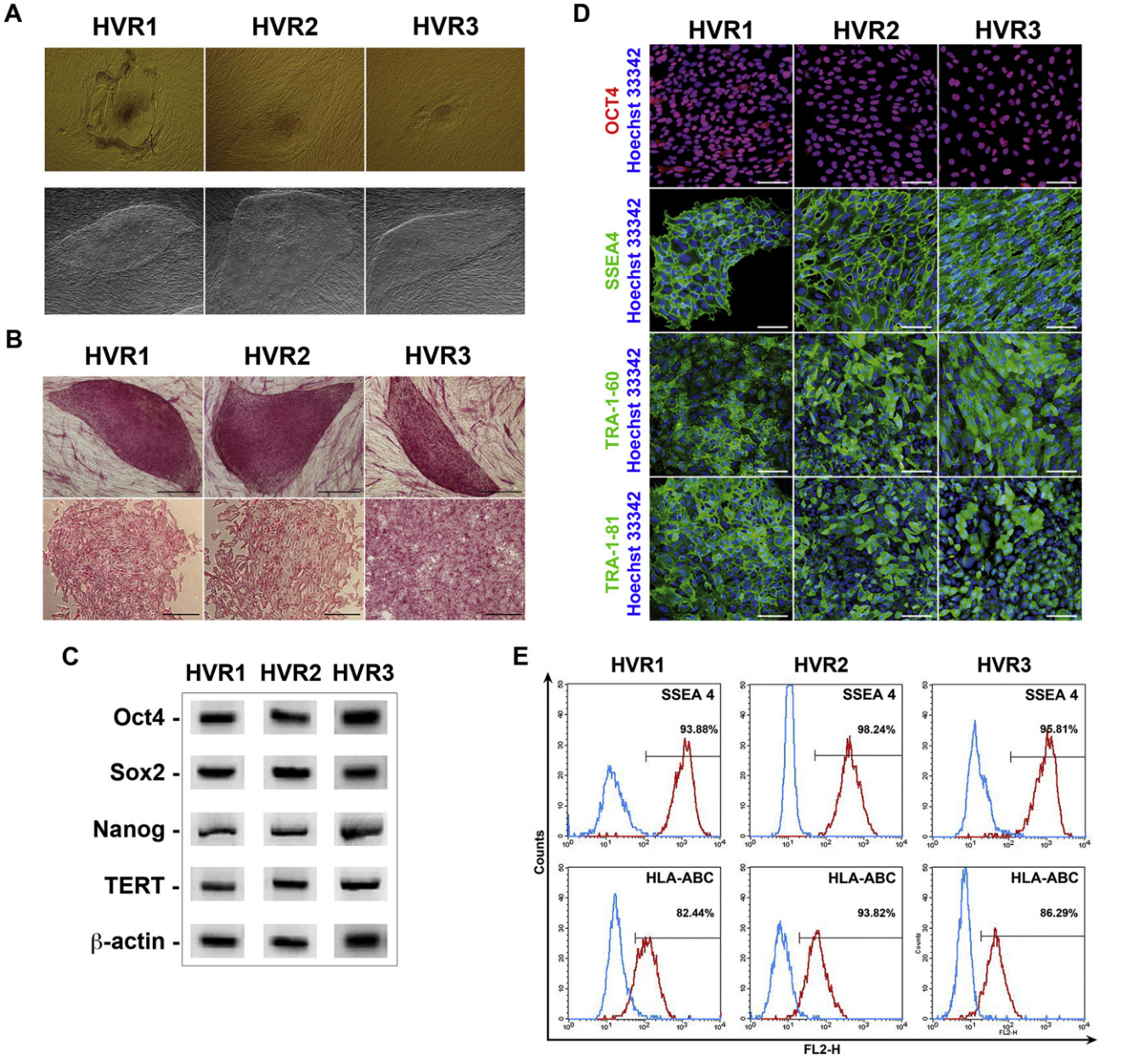

Fig. 1. Characterization of the HVR1, HVR2 and HVR3 cell lines. A) ICMs from HVR1, HVR2 and HVR3 after 12 days in culture and HVR3 ICM after 8 days in culture on human newborn fibroblast feeder cells (hFFs). Representative bright-field of human embryonic stem cells (hESCs) colonies cultured on hFFs (bottom). B) HVR1, HVR2 and HVR3, cultured on hFFs (top) or on BD Matrigel™ (bottom), are positive for alkaline phosphatase staining. Scale bar: 200 μm. C) RT-PCR analysis of Oct4, Sox2, Nanog and TERT genes expression in undifferentiated hESC lines HVR1, HVR2 and HVR3. β-actin was used as control. D) Immunofluorescence detection of OCT4, SSEA4, TRA-1-60 and TRA-1-81 expression in HVR1, HVR2 and HVR3 cell line, nuclei were stained with Hoechst 33342. The scale bar: 50 μm. E) Flow cytometry analysis indicating the expression of SSEA4 and HLA-ABC markers (red histograms). Blue histograms indicate cells labelled with fluorescent-conjugated isotype-matched antibodies.