Establishment of 2 control and 2 hPSC cell lines constitutively expressing the Notch ligand DLL4

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ARTICLE INFO

Article history:
Received 17 May 2017
Received in revised form 27 June 2017
Accepted 7 July 2017
Available online 10 July 2017

The Notch ligand DLL4 has key roles during embryonic development of different tissues, but most of the data comes from animal models. Here we describe the generation and characterization of 2 human Pluripotent Stem Cell (hPSC) lines that overexpress DLL4, as well as the two corresponding control hPSC lines. DLL4 expression can be detected at the mRNA and protein level, and does not affect the pluripotency of the cells. These hPSC lines can be used to study the role of DLL4 during human embryonic development.

Resource table.

| Unique stem cell lines identifier | - W Ae009-A-5  
| Alternative names of stem cell lines | - W Ae009-A-6  
| - GENYOi001-A-1  
| - GENYOi001-A-2  
| - PBMC1-iPS4F1 NEO (GENYOi001-A-1)  
| - PBMC1-iPS4F1 DLL4 (GENYOi001-A-2)  
| Institution | GENYO - Centre for Genomics and Oncological Research - Pfizer/University of Granada/Junta de Andalucía  
| Contact information of distributor | Verónica Ramos veronica.ramos@genyo.es  
| Contact information of distributor | Verónica Ayllón veronica.ayllon@genyo.es  
| Type of cell lines | iPSCs and hESCs  
| Origin | Human  
| Cell Source | The original hESC line is the H9 (WA09) from Wicell. The original iPSCs PBMC1-iPS4F1 has been derived from Peripheral Blood Mononuclear Cells  
| Method of reprogramming | The original iPSCs PBMC1-iPS4F1 was generated using non-integrative Sendai virus  
| Multiline rationale | For each cell type, we present the data for the control cells transduced with the empty vector plasmid (NEO) and the cells transduced with a vector containing the human DLL4 gene.  
| Gene modification | YES  
| Type of modification | Transgene expression, selected using Neomycin resistance  

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Resource utility

The Notch ligand DLL4 participates in embryonic development of cardiovascular and neural systems (Duarte et al., 2004; Rocha et al., 2009). Most of this knowledge comes from animal models, so these hPSCs overexpressing DLL4 are useful tools to study the role of DLL4 during human development.

Resource details

We transduced the iPSC line PBMC1-iPS4F1 and the hESC line H9 (Thomson et al., 1998) with lentiviruses expressing either a control vector (containing only the Neomycin resistance) or a vector containing the cDNA of the human DLL4 gene (Supplementary Fig. S1A). After selection with Neomycin for 10 days we obtained resistant colonies that were grown and expanded, resulting in the generation of four cell lines, all them representing a mixed population or pools: H9 NEO (WAe009-A-5), H9 DLL4 (WAe009-A-6), PBMC1-iPS4F1 NEO (GENYOi001-A-1)
Table 1
Summary of lines.

<table>
<thead>
<tr>
<th>iPSC line</th>
<th>Abbreviation in figures</th>
<th>Gender</th>
<th>Age</th>
<th>Ethnicity</th>
<th>Genotype of locus</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>H9 NEO (WAe009-A-5)</td>
<td>H9 NEO</td>
<td>Female</td>
<td>Embryo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H9 DLL4 (WAe009-A-6)</td>
<td>H9 DLL4</td>
<td>Female</td>
<td>Embryo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBMC1-IPS4F1 NEO (GENYOi001-A-1)</td>
<td>PBMC1-IPS4F1 NEO</td>
<td>Female</td>
<td>39 years</td>
<td>Spanish Caucasian</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBMC1-IPS4F1 DLL4 (GENYOi001-A-4)</td>
<td>PBMC1-IPS4F1 DLL4</td>
<td>Female</td>
<td>39 years</td>
<td>Spanish Caucasian</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. The specific instructions for a Lab Resource: Multiple Stem Cell Lines say "No figure legend. Reference to images included in Resource details section".
and PBMC1-ipS4F1 DLL4 (GENYOi001-A-2) (Table 1). The identity of the cell lines was confirmed by Short Tandem Repeat (STR) profiling. The cells are routinely tested and are mycoplasma free.

We confirmed that the DLL4 hPSC lines expressed higher amounts of DLL4 mRNA than control NEO cells by quantitative PCR (Fig. 1A) and semi-quantitative PCR (Fig. 1B). We detected cell surface expression of DLL4 protein in our DLL4 overexpressing hPSC by flow cytometry (Fig. 1C), while it is barely expressed in undifferentiated hPSCs (Ayllón et al., 2015). H9 DLL4 cells show a homogenous DLL4 expression at the membrane in most cells, while ~40% of PBMC1-ipS4F1 cells express DLL4 at the membrane, reflecting the mix population nature of the cell lines. Another reason for this heterogeneity could be that DLL4 can localize at either the plasma membrane or intracellularly. By immunohistochemistry, that detects total DLL4 expression, we confirmed that both DLL4 over-expressing cell lines show a rather homogenous staining for DLL4 after EB differentiation (Supplementary Fig. S1C). The four cell lines present the typical morphology of pluripotent stem cells, growing in compact colonies, with tightly packed cells (Supplementary Fig. S1B) and are positive for alkaline phosphatase activity (Fig. 1D). They express the pluripotency markers SSEA4, TRA-1-81, TRA-1-60 and OCT3/4, and OCT4, REX1, NANOG and SOX2, assessed by PCR (Fig. 1F).

The cell lines H9 Neo and H9 DLL4 have a normal karyotype 46,XX, but 20% of H9 DLL4 cells present a trisomy of chromosome 12 (47,XX,+12). This trisomy has already been described as a common event in hESCs (Catalina et al., 2008). PBMC1-ipS4F1 NEO and PBMC1-ipS4F1 DLL4 present the abnormal karyotype 46,XX,+der(1)(q11;q11),-14, suggesting that the alteration was already present in the parental cell line (Supplementary Fig. S1D). We generated embryoid bodies (EBs) with all four cell lines and allowed them to differentiate spontaneously for 20–23 days. As it is shown in Fig. 1G, all 4 cell lines were capable of differentiation to the three germ layers, with all samples showing positive staining for the markers cytokeratin (endoderm), β-III tubulin (ectoderm), and vimentin (mesoderm), as assessed by immunohistochemistry (Table 2).

In summary, we have successfully established hPSC lines that stably overexpress the Notch ligand DLL4. Neither the expression of Neomycin resistance or DLL4 gene has affected the pluripotency of the cell lines. Therefore, these cell lines are now available to study how DLL4 regulates human embryonic development, with a special interest on its role during cardiovascular development and specification and commitment of neural progenitors.

**Materials and methods**

**Generation of H9 Neo, H9 DLL4, PBMC1-ipS4F1 NEO and PBMC1-ipS4F1 DLL4 cell lines**

We used the lentiviral vector pRRL-EF1a-PGK-Neo to clone the cDNA of the human DLL4 gene under the control of the EF1α promoter (Supplementary Fig. S1A). Viral particles pseudotyped with VSV-G were generated on 293 T cells by calcium-phosphate transfection protocol. The parental cell lines PBMC1-ipS4F1 and H9 were infected overnight on the day of passage with the lentivirus in the presence of 8 µg/ml polybrene and 10 µM Y-27632 (Sigma-Aldrich). After 48 h, the transfected cells were selected with 100 µg/ml Neomycin (Calbiochem) for 10 days. The cells are routinely grown on Matrigel-coated flasks using E8 medium.

**Flow cytometry analysis**

We dissociated the colonies using Trypke Express (Life Technologies). The cells were resuspended in FACS buffer (5% FBS, 2 mM EDTA in PBS) and incubated with the specific primary antibody for 15 min at room temperature, using an isotype-match antibody as negative control. For Oct3/4 staining, we used the A and B Fix & Perm Solutions (Invitrogen). After Oct3/4 primary antibody incubation, the cells were incubated with FITC-conjugated secondary antibody (BD Bioscience). Then, the cells were washed with FACS buffer and stained with the life probe 7-aminonaphthomycine D (7-AAD) (BD Bioscience) for 5 min at RT. We analyzed the cells in a FACS Verse flow cytometer using FACS Diva™ software program (BD Bioscience). The antibodies used are listed on Table 3.

**Table 2** Characterization and validation.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Test</th>
<th>Result</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>Photography</td>
<td>Normal appearance of packed colonies</td>
<td>Supplementary Fig. S1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>panel B</td>
</tr>
<tr>
<td>Phenotype</td>
<td>PCR Alkaline phosphatase activity</td>
<td>All cell lines express OCT4, REX, NANOG and SOX2</td>
<td>Fig. 1 panel F</td>
</tr>
<tr>
<td></td>
<td>Flow cytometry</td>
<td>All cell lines express the pluripotency markers SSEA4, TRA-1-81, TRA-1-60 and OCT3/4 at levels that range between 30–96%</td>
<td>Fig. 1 panel E</td>
</tr>
<tr>
<td>Genotype</td>
<td>PCR Flow cytometry</td>
<td>DLL4 cell lines overexpress DLL4 mRNA</td>
<td>Fig. 1 panels A and B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DLL4 cell lines express the protein at the cell surface</td>
<td>Fig. 1 panel C</td>
</tr>
<tr>
<td></td>
<td>Karyotype (G-banding) and resolution</td>
<td>All karyotypes have a resolution of 400–550</td>
<td>Supplementary Fig. S1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>panel C</td>
</tr>
<tr>
<td>Identity</td>
<td>Microsatellite PCR (mPCR) STR analysis</td>
<td>All cell lines were tested for 16 loci, and they perfectly matched those of the parental cell lines</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Submitted in archive with journal</td>
</tr>
<tr>
<td>Mutation analysis (IF APPLICABLE)</td>
<td>Sequencing Southern Blot OR WCS</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Microbiology and virology</td>
<td>Mycoplasma Mycoplasma testing by RT quantitative PCR: Negative</td>
<td>NA</td>
<td>NA</td>
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<td></td>
<td></td>
<td></td>
<td>Submitted in archive with journal</td>
</tr>
<tr>
<td>Differentiation potential</td>
<td>Embryoid body formation n</td>
<td>Proof of three germ layers formation by immunohistochemistry against markers vimentin, cytokeratin and β-III tubulin</td>
<td>Fig. 1 panel G</td>
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<tr>
<td>Donor screening (OPTIONAL)</td>
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<tr>
<td>Genotype additional info (OPTIONAL)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
RT-PCR and quantitative PCR

RNA was extracted using High Pure RNA Isolation Kit (Roche) following manufacturer’s instructions. cDNA from total RNA was synthesized using the First-Strand cDNA Synthesis Kit (GE Healthcare) following manufacturer’s instructions. The quantitative PCR was performed using SYBR green (BioRad) in an ABI 7900HT instrument. We performed the PCRs using the primers listed on Table 3.

Alkaline Phosphatase activity assay

The cells were seeded on 12-well plates coated with Matrigel. After 5 days in culture, we tested the alkaline phosphatase enzymatic activity using the Alkaline Phosphatase Detection Kit (Merck-Millipore) following manufacturer’s instructions.

Embryoid body (EB) formation

One to two days before reaching confluence, 1 ml of Matrigel 1:6 (diluted in KO-DMEM) was added to the culture. The confluent culture was scrapped and cultured in ultra-low attachment wells (Corning) to induce EB formation. EBs were cultured for 20–23 days with E6 medium without bFGF2 for spontaneous differentiation to the three germ layers. Medium changes were performed every 2–3 days. At the end of differentiation, EBs were harvested, fixed and embedded in paraflin for histological examination by immunohistochemistry. The antibodies used are listed on Table 3.

Karyotype analysis

Chromosomal analysis was performed by GTG-banding analysis at the Andalusian Public Health System Biobank, Spain, according to the International System Cytogenetics Nomenclature recommendations. Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2017.07.008.

Acknowledgments

We are very grateful to our colleagues at the Gene Regulation, Stem Cells & Development Lab for their help and critical revision of this work. This work was supported by the ISCIII-FEDER P14/01412 to V.R.-M and the Andalusian Public Health System PI-0030-2014 to V.R.-M. J.V.R is supported by the Postdoctoral Juan de la Cierva Program (Spanish Ministry of Economy and Competitiveness, JCI_2012_12666). V.R.-M. is funded by the Andalusian Public Health System PI-0030-2014 to RM. V.M. is funded by the Postdoctoral Juan de la Cierva Program (Spanish Ministry of Economy and Competitiveness, JCI_2012_12666). J.D.-R. is supported by the Fundacion Inocente Inocente (FII2015-212) and the Asociación de Madres y Padres de Niños Oncológicos de Granada (AUPA).

References


Depósito de la línea celular PBMC1-iPS4F1 DLL4.

Figura Suplementaria:
Expresión del marcador de pluripotencia SSEA3 por citometría de flujo.