Reprogramming of Human Peripheral Blood Mononuclear Cell (PBMC) from a patient suffering of a Werner syndrome resulting in iPSC line (REGUi003-A) maintaining a short telomere length

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Lab resource: Stem Cell Line

Reprogramming of Human Peripheral Blood Mononuclear Cell (PBMC) from a patient suffering of a Werner syndrome resulting in iPSC line (REGUi003-A) maintaining a short telomere length

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ABSTRACT

Werner syndrome (WS) is a rare human autosomal recessive disorder characterized by early onset of aging-associated diseases, chromosomal instability, and cancer predisposition, without therapeutic treatment solution. Major clinical symptoms of WS include common age-associated diseases, such as insulin-resistant diabetes mellitus, and atherosclerosis. WRN, the gene responsible for the disease, encodes a RECQL-type DNA helicase with a role in telomere metabolism. We derived a stable iPSC line from 53 years old patient's PBMC, with a normal karyotype, but exhibiting a short telomere length, as a major aspect of the cellular phenotype involved in the pathology.

Resource utility

Werner syndrome is an heterogeneous genetic disease due to mutations in WRN helicase (Uhrhammer et al., 2006). The specific mutation of this patient, c.3789C > G hmz p.(Tyr1263*), generating a premature stop codon, does result in the absence of a nuclear translocated protein. Consequently, this iPSC line maintains a short telomere constituting a promising tool for disease modelling.

Resource details

PBMC were obtained from a (53)-years-old man harbouring a specific mutation in the WRN helicase (resource utility and Fig. 1A-Scheme for the functional domains of the Werner protein and position of the mutation), triggered in proliferation/differentiation toward the erythroid lineage and reprogrammed after a transient expression of the four reprogramming factors OCT3/4, SOX2, KLF4, and C-MYC using the integration-free Sendai virus gene-delivery method. The resulting WRN iPSC line had a normal morphology (Fig. 1B-Characterization of WRN iPSC colonies, by morphology (bright field picture), alkaline phosphatase staining and by Immuno-fluorescence staining after 10 passages analyzed by microscopy and Flow Cytometry using specific pluripotency markers). Expression of pluripotency markers was revealed by immunocytochemistry staining analyzed by fluorescence microscopy and cytometry (Fig. 1B). The WRN iPSC identity line was confirmed and compared with parental cells by STR analysis (provided as Supplementary Table 1) and the presence of the disease-associated mutation in the WRN gene by DNA sequencing (Fig. 1C-Characterization of the WRN mutation by Sanger sequencing of the genomic DNA allowing the confirmation of the homozygous mutation detected in the WRN gene of the patient (c.3789C > G hmz p.(Tyr1263*) A red square is around the new guanine). The WRN iPSC line exhibited a normal and stable diploid karyotype (46,XY) and absence of additional CNV when compared to the parental cells (Fig. 1D-Karyotype analysis performed on Werner iPSC line showing no global genomic alteration and Supplementary Fig. 1). Pluripotency was assessed by the ability of the WRN iPSC to...
Fig. 1.
differentiate into the three germ layers by the formation of teratoma in immune-compromised mice (Fig. 1E-Teratoma assay on the WRN iPSC line analyzed after HES staining of histological sections) and by transcriptomic analysis of pluripotency genes comparing fibroblasts/embryoid bodies and different iPSC lines (Fig. 1F-Heatmap of WRN iPSC line transcriptome, analyzed by supervised clustering on genes involved in pluripotency: The list of genes involved in pluripotency was described in Guenther et al., 2015 and transcriptomic analysis was performed on fibroblasts BJ (Bj BJ), fibroblasts from GEO Samples GSM1566208 (Fibro CCA and CCB), iPSC derived from BJ fibroblasts with 4 reprogramming factors OSKM or 6 factors OSKMN (Lapasset et al., 2011), respectively (iPSC BJ 4F and iPSC BJ 6F) and the corresponding Embryoid Bodies harvested at 15 days of differentiation (EB iPSC 4F and EB iPSC 6F, iPSC obtained from the WS patient (WRN iPSC), the human embryonic pluripotent stem cell line H9 (hES H9) and Fig. 1G-Heatmap of WRN iPSC line transcriptome, analyzed by supervised clustering on genes involved in teratomes maintenance: The list of genes was from Vaziri et al., 2010 and transcriptomic data used were as in Fig. 1F). Telomere length was evaluated by QFISH shorter than a control iPSC BJ line, which is consistent with the absence of functional WRN protein. (Fig. 1H-Telomere length in Werner iPSC line, evaluated by Q FISH analysis using specific fluorescent PNA is smaller than in iPSC derived from fibroblast BJ).

Materials and methods

Reprogramming PBMC into iPSCs

PBMC were isolated from blood using standard procedures and were cultured in SFEM II (StemSpan™ SFEM II Stemcell, cat.# 09605) medium with cytokines (StemSpan™ Erithroid Expansion Supplement (100 ×) Stemcell, Catalog cat#02692) at a density of 5.10⁵ cells per ml, during 7 days. The cells were transduced by Sendai-virus using the CytoTune-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific, cat#A34546), delivered at an MOI of 10⁻⁶ (KOS uroplakin III, no. A15169-01), according to the manufacturer’s instructions. For iPSC induction, PBMC were transduced by Sendai virus with 4 reprogramming factors OSKM or 6 factors OSKMN (Lapasset et al., 2011), respectively (IPSC BJ 4F and iPSC BJ 6F) and the corresponding Embryoid Bodies harvested at 15 days of differentiation (EB iPSC 4F and EB iPSC 6F, iPSC obtained from the WS patient (WRN iPSC), the human embryonic pluripotent stem cell line H9 (hES H9) and Fig. 1G-Heatmap of WRN iPSC line transcriptome, analyzed by supervised clustering on genes involved in teratomes maintenance: The list of genes was from Vaziri et al., 2010 and transcriptomic data used were as in Fig. 1F). Telomere length was evaluated by QFISH shorter than a control iPSC BJ line, which is consistent with the absence of functional WRN protein. (Fig. 1H-Telomere length in Werner iPSC line, evaluated by Q FISH analysis using specific fluorescent PNA is smaller than in iPSC derived from fibroblast BJ).

iPSC culture and genomic DNA extraction

iPSCs were cultured in SFEM II Stem cell medium supplemented with cytokines (StemSpan™ Erithroid Expansion Supplement (100 ×) Stemcell, Catalog cat#02692) at a density of 5.10^5 cells per ml, during 7 days. The cells were transduced by Sendai-virus using the CytoTune-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific, cat#A34546), delivered at an MOI of 10⁻⁶ (KOS uroplakin III, no. A15169-01), according to the manufacturer’s instructions. For iPSC induction, PBMC were transduced by Sendai virus with 4 reprogramming factors OSKM or 6 factors OSKMN (Lapasset et al., 2011), respectively (IPSC BJ 4F and iPSC BJ 6F) and the corresponding Embryoid Bodies harvested at 15 days of differentiation (EB iPSC 4F and EB iPSC 6F, iPSC obtained from the WS patient (WRN iPSC), the human embryonic pluripotent stem cell line H9 (hES H9) and Fig. 1G-Heatmap of WRN iPSC line transcriptome, analyzed by supervised clustering on genes involved in teratomes maintenance: The list of genes was from Vaziri et al., 2010 and transcriptomic data used were as in Fig. 1F). Telomere length was evaluated by QFISH shorter than a control iPSC BJ line, which is consistent with the absence of functional WRN protein. (Fig. 1H-Telomere length in Werner iPSC line, evaluated by Q FISH analysis using specific fluorescent PNA is smaller than in iPSC derived from fibroblast BJ).

Flow cytometry analysis

BD Stemflow Human Pluripotent Stem Cell Transcription Factor Analysis Kit. Cells were analyzed on a CANTO II Becton Dickinson and analysis was made with Flow-JO. Results are presented in Fig. 1B and antibodies used are in Table 1.

Immunofluorescence

Cells grown on coverslips were fixed in 4% paraformaldehyde in PBS and labelled overnight at room temperature, after 0.1% Saponin permeabilization in the blocking buffer (5% goat serum) for 60 min according to the standard protocol of StemLight™ Pluripotency Antibody Kit (Cell Signaling, no.9656). Antibodies panel, listed in Table 1, included: Oct-4A, Sox2, Nanog, SSEA4, TRA-1-60, TRA-1-81. Appropriate fluorochrome-conjugated anti primary antibodies with Alexa Fluor® 488 and Alexa Fluor® 555 dyes were applied 60 min. DNA was stained with DAPI (ImmunoChemistry, no.6244) 15 min and cover-slips mounted in Vectashield (Vector, no.H-1400). Image acquisition was performed with an Axio Imager Z1(ZEISS) Apotome, X10 objective (Table 2).

Transcriptomic analysis

Total RNA isolation was performed using the RNaseasy mini kit (Qiagen) according to manufacturer’s instructions. RNA was hybridized on a Affymetrix GeneChip Human Genome U133 Plus 2.0 array (shown in Fig. 1F and G).

Teratoma formation

The differentiation potential was performed by in vivo teratoma derivation. Clusters corresponding to approximately 3 × 10⁶ of iPSC cells were injected into anesthetized NOD SCID gamma (NOD.CgPrkdcscidIl2rg tm1Wjl/SzJ). Mice were transplanted subcutaneously in dorso-lateral area on both sides at 8 weeks old. After 4–8 weeks of latency and a 100% derivation efficiency, teratomas were fixed, embedded in paraffin blocks, stained by HES and analyzed by a pathologist for the presence of structures within the 3 embryonic germ layers (shown in Fig. 1G).
### Table 1: 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>Visual record of the line: normal colonies</td>
<td>Fig. 1, panel B</td>
</tr>
<tr>
<td>Phenotype</td>
<td>Qualitative analysis (Immunofluorescence and Flow Cytometry)</td>
<td>Oct4, Nanog, Sox2, Nanog, SSEA4, Tra-1-81, Tra-1-60</td>
<td>Fig. 1, panel B</td>
</tr>
<tr>
<td>Genotype</td>
<td>Quantitative analysis (Flow Cytometry)</td>
<td>Oct4/3:4:100%, Nanog: 100%, SSEA-4: 100%, SOX-2: 100%</td>
<td>Fig. 1, panel B</td>
</tr>
<tr>
<td>Identity</td>
<td>CVN microarray</td>
<td>46XY, Resolution 450 No</td>
<td>Fig. 1, panel D</td>
</tr>
<tr>
<td>Mutation analysis (IF APPLICABLE)</td>
<td>STR analysis</td>
<td>DNA profiling</td>
<td>Not performed</td>
</tr>
<tr>
<td>Microbiology and virology</td>
<td>Mycoplasma</td>
<td>Tested by luminescence, as Negative</td>
<td>Supplementary Table 1</td>
</tr>
<tr>
<td>Differentiation potential</td>
<td>Teratoma formation</td>
<td>Detection of the presence of the three embryo germ layer by histochemistry (validated by a certified Anatomist-Histopathologist)</td>
<td>Fig. 1, panel E</td>
</tr>
<tr>
<td>Donor screening (OPTIONAL)</td>
<td>HIV 1 + 2 Hepatitis B, Hepatitis C</td>
<td>Negative by Elias analysis</td>
<td>Not shown but available with authors</td>
</tr>
<tr>
<td>Genotype additional info (OPTIONAL)</td>
<td>Blood group genotyping</td>
<td>Not performed</td>
<td>Not available</td>
</tr>
<tr>
<td></td>
<td>HLA tissue typing</td>
<td>Not performed</td>
<td>Not available</td>
</tr>
</tbody>
</table>

### Mycoplasma detection

Mycoplasma is detected with MycoAlert® Detection Kit (Lonza) according to manufacturer’s instructions (Supplementary Table 2).

### Key resources table

- **Unique stem cell line identifier**: REGU0003-A
- **Alternative name(s) of stem cell line**: WRN iPSC
- **Institution**: CHU Montpellier, Saint Eloi Hospital
- **Contact information of distributor**: Dr. Lemaire
- **Type of cell line**: iPSC
- **Origin**: human
- **Cell line repository/bank**: SAFE-iPS facility IRMB
- **Ethical approval**: CPP CHU MONTPELLIER. 2014-A00178-39
- **Associated disease**: Werner syndrome
- **Ethnicity**: Caucasian
- **Sex**: Male
- **Age**: 53
- **Cell Source**: blood
- **Clonality**: mixed
- **Method of reprogramming**: Sendai
- **Genetic Modification**: YES
- **Type of Modification**: Hereditary
- **Associated disease**: Werner syndrome
- **Gene/locus**: (NM_000553) Werner syndrome ATP-dependent helicase
- **Method of modification**: No
- **Name of transgene or resistance**: None
- **Inducible/constitutive system**: None
- **Date archived/stock date**: None
- **Additional origin info**: Age:53 Sex: Male Ethnicity: Caucasian

### Table 2: Reagents details-Werner iPSC.

#### Antibodies used for immunocytochemistry/flow-cytometry

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Dilution</th>
<th>Company Cat # and RRID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct-4A Rabbit mAb (Clone C30A3) IgG</td>
<td>1:200</td>
<td>Cell signaling technology cat# 2840, RRID:AB_2167691</td>
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<tr>
<td>Sox2 XP® Rabbit mAb (Clone D6D9) IgG</td>
<td>1:200</td>
<td>Cell signaling technology cat# 3579, RRID:AB_2195767</td>
</tr>
<tr>
<td>Nanog XP® Rabbit mAb (Clone D7364) IgG</td>
<td>1:200</td>
<td>Cell signaling technology cat# 4903, RRID:AB_10559205</td>
</tr>
<tr>
<td>TRA-1-60(S) Mouse mAb (Clone TRA-1-60(S)) IgM</td>
<td>1:200</td>
<td>Cell Signaling Technology Cat# 4755, RRID:AB_1264259</td>
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<tr>
<td>TRA-1-81 Mouse mAb (Clone TRA-1-81) IgM</td>
<td>1:200</td>
<td>Cell Signaling Technology Cat# 4746, RRID:AB_2119059</td>
</tr>
<tr>
<td>PE Mouse anti-human Nanog (Clone: N31–355)</td>
<td>1:5</td>
<td>BD Biosciences Cat# 560791, RRID:AB_1937313</td>
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<tr>
<td>PerCP-CyTm 5.5 Mouse anti-Oct3/4 (Clone: 40/Oct-3)</td>
<td>1:5</td>
<td>BD Biosciences Cat# 560794, RRID:AB_1937313</td>
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<tr>
<td>Alexa Fluor® 647 Mouse anti-Sox2 (Clone: 245610)</td>
<td>1:5</td>
<td>BD Biosciences Cat# 560301, RRID:AB_1645308</td>
</tr>
<tr>
<td>Alexa Fluor® 647 Mouse anti-SSEA-4 (Clone: MCB13–70)</td>
<td>1:5</td>
<td>BD Biosciences Cat# 560796, RRID:AB_2033991</td>
</tr>
<tr>
<td>PE Mouse IgG1, x Isotype Control (Clone MOPC-21)</td>
<td>1:5</td>
<td>BD Biosciences Cat# 554121, RRID:AB_396989</td>
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<tr>
<td>PerCP-Cy5.5 Mouse IgG1, x Isotype Control (Clone: X40)</td>
<td>1:5</td>
<td>BD Biosciences Cat# 347202, RRID:AB_400265</td>
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<tr>
<td>Alexa Fluor® 647 Mouse IgG2a, x Isotype Control (Clone: MOPC-173)</td>
<td>1:5</td>
<td>BD Biosciences Cat# 558020, RRID:AB_1996989</td>
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<tr>
<td>Secondary Antibody Alexa Fluor® 488 conjugate Goat anti-Rabbit IgG</td>
<td>1:400</td>
<td>Invitrogen-thermo fisher scientific cat# A-11034, RRID:AB_2576217</td>
</tr>
<tr>
<td>Secondary Antibody Alexa Fluor® 555 conjugate Goat anti-Rabbit IgG</td>
<td>1:400</td>
<td>Invitrogen-thermo fisher scientific cat# A-21424, RRID:AB_141780</td>
</tr>
</tbody>
</table>

#### Primers

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<th>Target</th>
<th>Forward/Reverse primer (5′-3′)</th>
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<tbody>
<tr>
<td>Werner mutation (Sanger sequencing)</td>
<td>WERNER mutation 5′TGAGCTGCCCTATATAAAGGGAA3′, 5′TGCGCACAATCAAACCTTGCTG3′</td>
</tr>
</tbody>
</table>
Declaration of Competing Interest

None.

Acknowledgements

Research in the laboratory of Jean-Marc Lemaitre was supported by INSERM, the University of Montpellier, the CHRU Montpellier/SAFE-iPSC facility (INGESTEM consortium Infrastructure en Biology Santé), and by a Grant from Ligue National Centre le Contre le cancer “Équipe Labellisée (2015-2019)” and an AOI young researcher from CHU Montpellier.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2019.101515.

References


