Stem Cell Research 15 (2015) 595–597

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab resource: stem cell line

Generation of poikiloderma with neutropenia (PN) induced pluripotent stem cells (iPSCs)

Jason A. Mills [a,b,*], Kristin M. Hudock [c], Spencer K. Sullivan [e], Pamela Herrera [a], Lisa M. Sullivan [a], Paul Gadue [a,b,d], Deborah L. French [a,b,d]

[a] Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia, Philadelphia, PA, USA
[b] Center for Cellular and Molecular Therapeutics, The Children's Hospital of Philadelphia, Philadelphia, PA, USA
[c] Division of Pulmonary, Allergy, and Critical Care Medicine, The University of Pennsylvania School of Medicine, Philadelphia, PA, USA
[d] Department of Pathology and Laboratory Medicine, The University of Pennsylvania, Philadelphia, PA, USA
[e] Division of Hematology, The Children's Hospital of Philadelphia, Philadelphia, PA, USA

ARTICLE INFO

Article history:
Received 2 October 2015
Received in revised form 8 October 2015
Accepted 14 October 2015
Available online 16 October 2015

ABSTRACT

Poikiloderma with neutropenia (PN, Clericuzio-type poikiloderma with neutropenia) is a rare autosomal recessive disorder caused by biallelic mutations in the *USB1* gene (Alias *C16orf57* and *MPN1*). To date, there have been only 37 reported cases worldwide of this disorder that presents with neutropenia, early onset poikiloderma, respiratory infections, palmo-plantar hyperkeratosis, and skeletal defects. Here we described the generation of human induced pluripotent stem cell lines (PN1 and PN2) from the peripheral blood of a 1-year-old patient using the dox-inducible STEMCCA vector. This patient presented with bacteremia, pneumonia, and neutropenia. Analysis of bone marrow demonstrated normal cellularity with trilineage hematopoiesis and neutropenia.

Resource table:

Name of stem cell construct	PN-IPSCs
Institution	The Children's Hospital of Philadelphia
Person who created resource	Jason A. Mills
Contact person and email	Deborah L. French, frenchd@email.chop.edu
Date archived/stock date	2012–2013
Origin	Infant human peripheral blood mononuclear cells (PBMCs)
Type of resource	Induced pluripotent stem cell (iPS) (Fig. 1A,D,F)
Cell line	Cell line
Key transcription factors	OCT4, SOX 2, KLF4, cMYC
Authentication	Mutation and purity of cell line confirmed (Fig. 1B, C, E)
Link to related literature (direct URL links and full references)	Colombo, E.A. et al. Orphanet Journal of Rare Disease 2012: v7:p7. Patient #25 http://www.ojrd.com/content/7/1/7
Information in public databases	N/A

Resource details

Peripheral blood mononuclear cells (PBMCs) were collected from a patient carrying a mutation in *USB1* (c.693 + 1G>T), transduced with dox-inducible STEMCCA vectors overexpressing OCT4, SOX2, KLF4, and cMYC.

The mutation was validated in iPSCs by DNA sequencing and cDNA amplification of the truncated mRNA transcript. Verification of pluripotency was performed by flow cytometry and qRT-PCR of common undifferentiated markers.

G-banding was performed to verify normal 46, XX karyotype.

Teratoma analysis was performed to demonstrate all three embryonic germ layers.

1. Materials and methods

The recruitment of this human subject and iPSC generation for this manuscript was approved by The Children's Hospital of Philadelphia's IRB (IRB 09-007,042) and ethics committee. PBMCs were collected from a patient with poikiloderma with neutropenia, harboring a mutation in the *C16ORF57* gene, and reprogrammed as previously described (Colombo et al., 2012; Sommer et al., 2012). Briefly, 5 × 10^5 PBMCs were expanded in QBSF-60 media containing EPO (2 U/mL), IGF-1 (40 ng/mL), SCF (50 ng/mL), IL-3 (10 ng/mL), dexamethasone (1 μM), ascorbic acid (50 ng/mL), glutamine (1%) and penicillin/streptomycin (1%) for 7 days. Cells were transduced with a doxycycline-regulated human STEMCCA lentivirus expressing OCT4, SOX2, KLF4 and cMYC. After 24 h, 2 μg/mL of doxycycline (DOX) was added to the media and cells were placed in culture dishes containing irradiated mouse embryonic feeders (MEFs). At day 4 post-transduction, the media was changed to human embryonic stem cell media (HES) containing 5 ng/mL of bFGF + 2 μg/mL of DOX. The media was replenished every

* Corresponding author.

http://dx.doi.org/10.1016/j.scr.2015.10.006

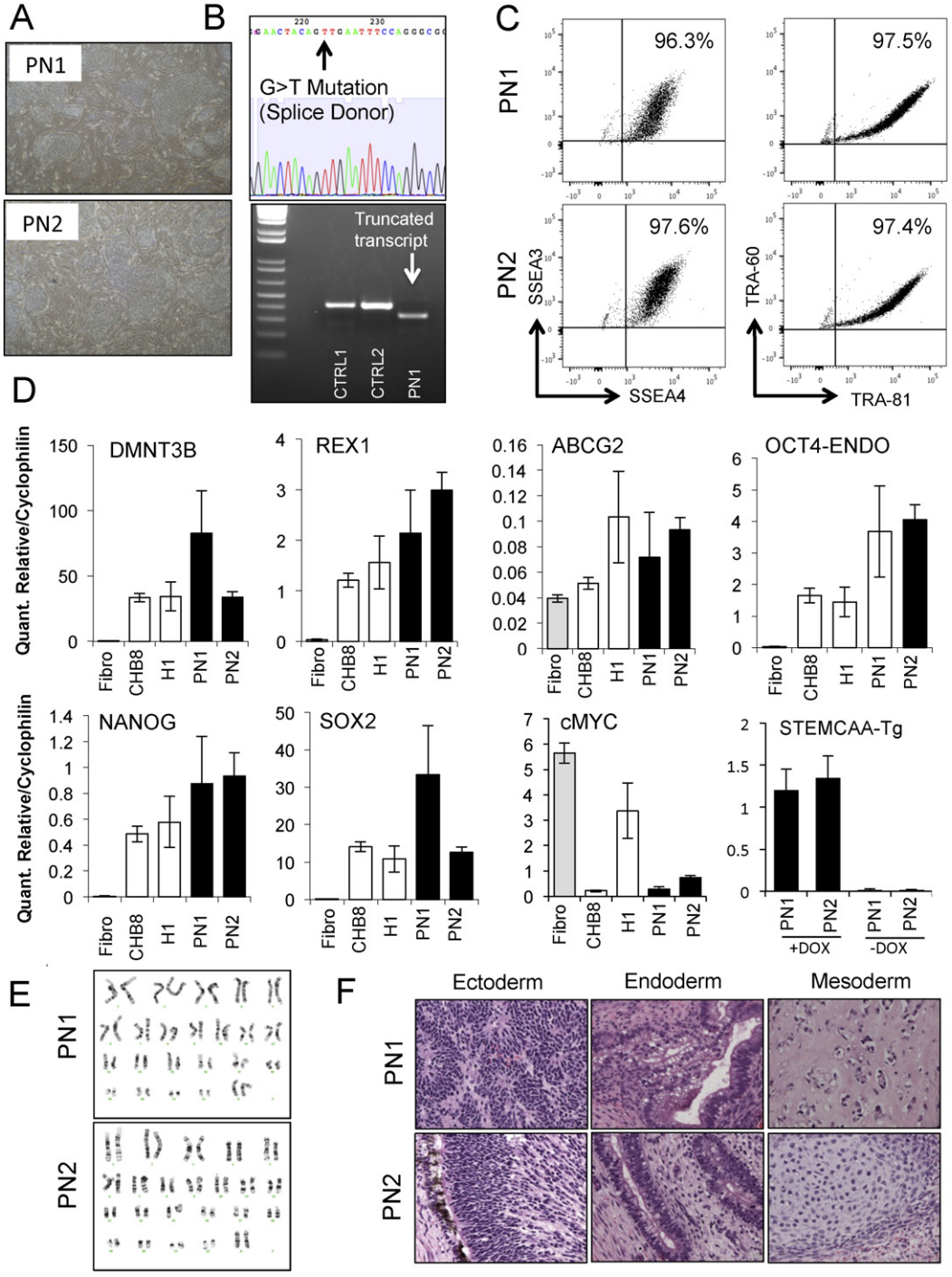

Fig. 1. Analysis of iPSCs generated from a poikiloderma with neutropenia (PN) patient. A. Morphology of PN-iPSC colony. B. The splicing mutation affects the donor splice site of IVS6, c693 + 1G>T, detected in the homozygous state and results in truncated cDNA. C. Representative flow cytometry analysis plots of undifferentiated markers: SSEA3, SSEA4, TRA-1-60, TRA-1-81. D. Quantitative PCR of relative gene expression of key pluripotency genes compared to fibroblast and embryonic stem cell lines (CHB8 and H9), and transgene silencing in the absence of doxycycline. E. Karyotype of PN-IPSCs derived from patient sample. F. Hematoxylin and eosin staining of teratoma sections demonstrating the potential for generation of all three germ layers.

2–3 days for 3 weeks. Cells were maintained in these conditions until uniform colonies were generated, and iPSC colonies were mechanically isolated and expanded on MEFs (Fig. 1A).

2. Mutation verification.

Genomic DNA was isolated from iPSCs using a blood and tissue extraction kit (Qiagen, Valencia, CA). About 50 ng of DNA was amplified using Platinum PCR Supermix high fidelity (Invitrogen) with primers: Forward: CCTCCTTCCACAGATTCTTC and Reverse: GTTCCTCCATCTCA GCCTG, (Clericuzio et al., 2011) using thermocycler conditions: 95C for 10 min, 94C for 30 s and 60C for 15 s, and 72C for 30 s for 35 cycles, then a 4C hold. Amplicons were sequenced, and electropherograms were analyzed with the APE plasmid editor using the wild type sequence of the *C16orf57* gene [ENSG00000103005] as reference.

3. Flow cytometry

Analysis of cells was performed on a Cantos flow cytometer (Becton Dickinson, San Jose, CA). Human PN iPSCs were disassociated to single

cells using Accutase and were analyzed for cell surface markers SSEA3-488, SSEA4-647, Tra-1-60-PE and Tra-1-81-APC (Biolegend). Flow cytometric analysis was performed using the FlowJo (Treestar, San Carlos, CA) software program.

4. RT-PCR and quantitative PCR

Total RNA was collected using the RNeasy micro kit (Qiagen) and treated with on-column RNase-free DNase. The first strand synthesis of cDNA was performed using 1 μg of total RNA, according to manufactures specification. RT-qPCR was performed on the LightCycler-480 (Roche-Genentech, South San Francisco, CA). All experiments were performed in triplicate using SYBR-Green qPCR supermix (Life technologies, Grand Island, NY) according to manufacturer's instructions. Primers for all genes were prepared by IDT (www.idt.dna.com), as previously published (Mills et al., 2013). Dilutions of genomic DNA were used as standards ranging from 1 ng to 100 ng/μl, and were used to determine PCR efficiency and relative gene expression compared to the housekeeping gene Cyclophilin G. Verification of the truncated transcript was performed using Platinum Blue Supermix, 50 ng of cDNA and primers: Forward: CCTCCTTCCACAGATTCTTC, Reverse: GTTCCTCCATCTCAGCCTG. PCR conditions: as above.

5. Teratoma formation

Teratoma assays were performed as previously described (Mills et al., 2013). Briefly, iPSCs (1×10^6 cells) were injected subcutaneously into the leg of a NOD/SCID mouse. Tumor growth was monitored weekly by palpation. Animals were sacrificed when the tumor size was at roughly 1 cm. The tumor was placed in 4% phosphate buffered formalin for 24 h and then embedded in paraffin. For histological analysis, the specimens were stained with hematoxylin and eosin (HE).

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