Stem Cell Research 17 (2016) 235–237

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Stem Cell Line

Generation of an induced pluripotent stem cell line from a patient with chronic myeloid leukemia (CML) resistant to targeted therapies

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ARTICLE INFO

Article history:
Received 6 July 2016
Received in revised form 23 July 2016
Accepted 2 August 2016
Available online 4 August 2016

ABSTRACT

Chronic myeloid leukemia (CML) is a clonal malignancy initiated by the occurrence of a t (9;22) translocation, generating Ph1 chromosome and BCR-ABL oncogene in a primitive hematopoietic stem cell (HSC). The resistance of HSC to targeted therapies using tyrosine kinase inhibitors remains a major obstacle towards the cure. We have generated an iPSC line from a patient with CML using leukemic CD34 + cells cryopreserved at diagnosis. Ph1 + CML cells were reprogrammed by non-integrative viral transduction. These iPSCs harboured Ph1 chromosome and expressed pluripotency hallmarks as well as BCR-ABL. Teratoma assays revealed normal differentiation after injection in immunodeficient mice.

Resource table.

Name of stem cell lines	PB32: Polyclonal PB32 iPSC line and three clones 32-1, 32-4 and 32-5
Institution	INSERM U935 and INGESTEM, Université Paris Sud
Person who created resource	G. Telliam, O. Feraud
Contact person and email	Ali G Turhan: turviv33@gmail.com, abenna@hotmail.fr
Date archived/stock date	September 1, 2013
Origin	Leukemic CD34 + cells
Type of resource	Biological reagent: induced pluripotent stem cell (iPSC) generated by Sendai-virus mediated pluripotency gene transfer
Sub-type	Cell lines
Key transcription factors	Oct4, Sox2, cMyc, Klf4
Authentication	Identity and purity of cell line, expressing the same BCR-ABL transcript as the peripheral blood cells of the patient
Information in public databases	None
Ethics	The project was approved by INSERM Ethical Committee

Resource details

Chronic myeloid leukemia (CML) is a malignant myeloproliferative disorder originating from a primitive hematopoietic stem cell leading to an expansion of the progenitors and differentiated myeloid cells in the peripheral blood (Chomel and Turhan, 2011). Despite the major progress obtained in this disease by the use of tyrosine kinase inhibitors, the most primitive stem cells are resistant to these therapies (Jiang et al., 2007; Chomel et al., 2011). We have generated iPSC from the cryopreserved diagnostic sample of a patient according to his clinical response to Imatinib, the first clinically licensed tyrosine kinase inhibitor. This 14-year old young patient exhibited a clear resistance to this drug with absence of molecular response after 18 months of starting therapy. There was no mutation in the ABL-kinase domain of the BCR-ABL gene. In 2005, given his young age and the availability of a HLA-compatible sibling, an allogeneic stem cell transplant was performed with success. In 2016, he remains in good health in complete haematological and molecular remission. An induced pluripotent stem cell (iPSC) line were generated using cryopreserved CD34 + leukemic cells collected at diagnosis by non-integrative transduction of Oct3/4, Sox2, Klf4, and cMyc (Takahashi et al., 2007). iPSC colonies growing on MEF stromal layers were morphologically identified after 2–4 weeks (Fig. 1A), individually plucked as well as a polyclonal stock and expanded. Besides the polyclonal PB32 cell line, 3 clones (PB32–1, PB32–4 and PB32–5) were expanded. All have been characterized for their pluripotency. They exhibited typical markers of pluripotency such as expression of SSEA4

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http://dx.doi.org/10.1016/j.scr.2016.08.001

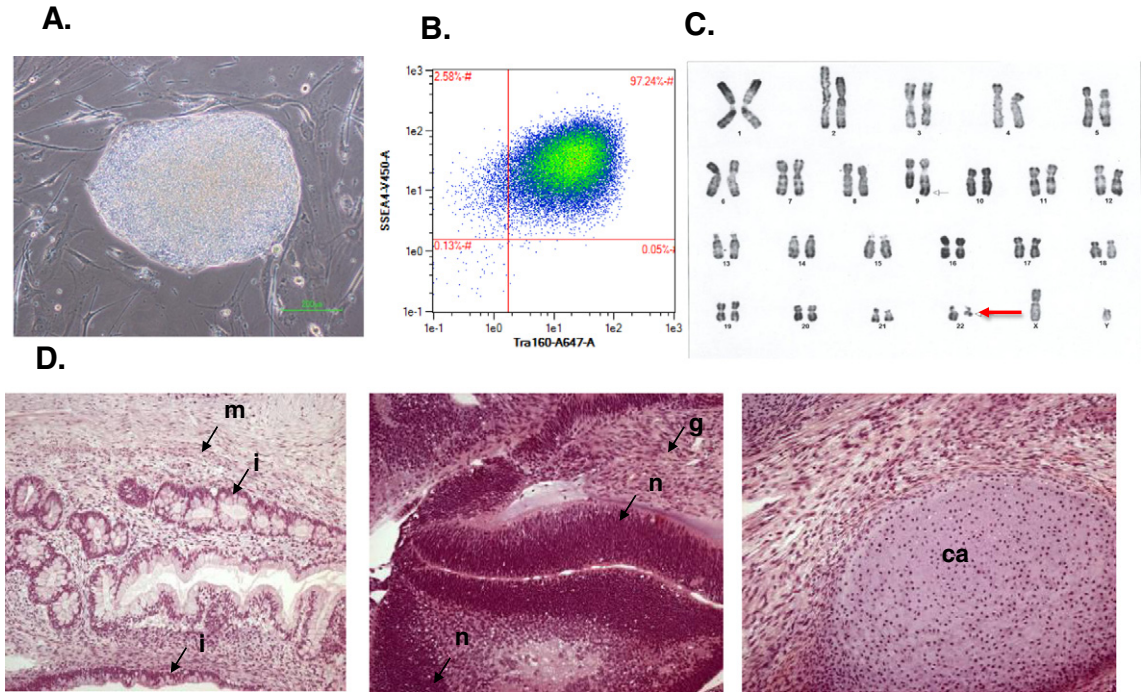

Fig. 1. Characterization of the PB32 Ph1-positive iPSC cell line. Panel A: Phase contrast imaging of PB32 colonies (polyclonal stock) grown on MEF stromal cells. Panel B: FACS evaluation of pluripotency markers using a double SSEA4 and TRA-1-60 staining confirming the presence of cell surface pluripotency markers on PB32 cells. Panel C: Cytogenetics analysis showing the presence of Ph1 chromosome (arrow). Panel D: Pathological analysis of a teratoma from PB32 iPSC, showing a normal ectodermal, endodermal and mesodermal differentiation. M: Muscle; i: Intestinal tissue with mucus formation; g: Glial tissue; n: Neural crest; ca: Cartilage.

and TRA-1-60 (Fig. 1B). Cytogenetics analysis demonstrated the presence of Ph1 chromosome (Fig. 1C). Teratoma assays showed the presence of normal differentiation towards endodermal, ectodermal and mesodermal layers (Fig. 1D).

Materials and methods

Generation of CML iPSC

Cryopreserved leukemic CD34+ peripheral blood mononuclear cells (PBMC) obtained at diagnosis were thawed and expanded in Myelocult™ medium (Stemcell Technologies) supplemented with 1% penicillin-streptomycin (Life technologies), hSCF (100 ng/ml), hFLT-3 (100 ng/ml), hIL-3 (20 ng/ml), hIL-6 (20 ng/ml) and hIL7 (20 ng/ml) (all of them from Peprotech) for 4 days. At day + 4, 2×10^5 CD34+ cells were then transduced overnight with Sendai viruses containing Oct3/4, Sox2, Klf4, and c-Myc (CytoTune®-iPS Sendai Reprogramming Kit, Life technologies), each of them at multiplicity of infection (MOI) of 15. Following the next 2 days, cell culture medium was changed daily and cells were resuspended in expansion medium after 5 minute centrifugation at 200 ×*g*. At day + 3, cells were recovered by centrifugation and plated on Mitomycin-C-treated mouse embryonic fibroblasts (MEF, CD1 strain) in expansion medium for 2 additional days. At day 6, half of the medium was changed to human pluripotent stem cell medium (hPSC medium) based on DMEM/F12 supplemented with 20% Knock Out Serum Replacer, 1 mM L-glutamine, 1% penicillin/streptomycin, 100 μM 2-mercaptoethanol (all of them from Life technologies) and 12.5 ng/ml basic FGF (Miltenyi Biotech). Then, the medium was changed daily with hPSC medium. 3–4 weeks after the lentiviral transduction, morphologically recognizable iPSC colonies appeared in culture for both cell lines. These colonies were manually picked and transferred to freshly Mitomycin-C-treated MEF for amplification.

After generation of a frozen stocks, iPSC could be cultured using either MEF co-culture technique or in feeder free conditions. The feeder cultures were performed on Mitomycin C-treated MEF layer as described above with passaging every 7 days using 1 mg/ml collagenase IV in DMEM/F12 (Life technologies); the feeder-free culture was performed on Geltrex™ (Life technologies) in Essential 8 medium (Life technologies) and 1% penicillin/streptomycin with passaging every 3–4 days using in DPBS (Life technologies) supplemented with 0.5 mM EDTA (Life technologies) and 1.8 mg/l NaCl (Sigma).

Karyotyping and expression of BCR-ABL

For conventional cytogenetics, cells grown in 60 mm plates (Corning) on mitomycin-C treated MEF for 5 days were cultured 2 h in hPSC medium supplemented with 100 ng/ml Colcemid™ (Life technologies). Cells were detached from the plate by 5 min treatment with 1 ml per plate of TrypLE™ Express (Life technologies) at 37 °C, 5% CO_2. After trituration with a P1000 micropipet, 9 ml of 0.075 M KCl (Life technologies) were added to the cell suspension followed by incubation at 37 °C during 20 min to obtain an osmotic shock. Cell suspension was then prefixed by the addition of 1 ml of fixative composed of Methanol/Acetic Acid (3:1) followed by 10 minute centrifugation at 130 ×*g*. Cell pellet was then resuspended in 10 ml of fixative and centrifuged 10 min at 130 ×*g* twice before a final resuspension in 10 ml of fixative. Cells were then spread on slides and stained with Giemsa. A minimum of 10 metaphases were captured and analysed using an automated imaging system (Meta Systems).

Pluripotency markers and BCR-ABL expression

For flow cytometry analysis, iPSC polyclonal stock and three clones PB32-1, PB32-4 and PB32-5 were recovered from Mitomycin-C inactivated MEF after incubation for 2 h in 1 mg/ml collagenase IV in DMEM/F12. After two cycles of washing/sedimentation with 10 ml DMEM/F12, colonies were washed once in Ca^{2+}/Mg2+ free PBS (Life technologies) and then dissociated into a single cell suspension by 10 min incubation in Hank's balanced enzyme-free cell dissociation buffer (Life Technologies). For pluripotency marker analysis, 1×10^5

cells were incubated in 10 μl PBS containing 1 μl V450-conjugated mouse monoclonal anti-SSEA-4 and 1 μl Alexa647-conjugated mouse monoclonal antibody anti-TRA1-60 (both from BD Biosciences) or conjugated isotype control. BCR-ABL expression was performed by both Q-RT-PCR and Western blots showing expression of BCR-ABL mRNA and protein in PB32 polyclonal iPSC and three clones (data not shown).

In vivo pluripotency analysis by teratoma formation

Teratoma assays were performed triplicate in order to analyze the development of potentially malignant tumor tissue within teratomas, by intramuscular injection of PB32 polyclonal iPSC and three clones in NSG mice (2.10^6 cells in 150 μl of Geltrex/DMEMEF12 1:1dilution). 10 weeks after injection, pathological analysis of teratomas was performed. This analysis revealed the presence of differentiated tissues including glial cells, neuroectodermal cells, intestinal cells and cartilage differentiation in all tumors with no evidence of malignancy, confirming the pluripotency of these cells (Fig. 1D).

Acknowledgements

This work was performed by the grant from INGESTEM national consortium.

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