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Lab Resource: Multiple Cell Lines

Generation and characterization of a human iPSC line SANi005-A containing the gray platelet associated heterozygous mutation p.Q287* in GFI1B

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ABSTRACT

Peripheral blood mononuclear cells were isolated from an individual harboring a heterozygous c.859C → T p.Q287* mutation in GFI1B, causing an autosomal dominant bleeding disorder, platelet type, 17 (BDPLT17). PBMCs were differentiated to erythroblasts and reprogrammed by lentiviral delivery of a self-silencing hOKSM polycistronic vector. Pluripotency of iPSC line was confirmed by expression of associated markers and by in vitro spontaneous differentiation towards the 3 germ layers. Normal karyotype confirmed the genomic integrity of iPSCs and the presence of disease causing mutation was shown by Sanger sequencing. The generated iPSCs can be used to study BDPLT17 pathophysiology and basic functions of GFI1B.

Resource table.

Unique stem cell line identifier	SANi005-A
Alternative name(s) of stem cell line	BEL20 cl.8
Institution	Sanquin, Amsterdam, The Netherlands
Contact information of distributor	e.vandenakker@sanquin.nl
Type of cell line	iPSC
Origin	peripheral blood (mobilized) mononuclear cells-derived EBL
Additional origin info	Age:79 Sex: female
Cell source	Erythroblast
Method of reprogramming	Lentivirus
Genetic modification	NO
Type of modification	c.859C → T p.Q287*
Associated disease	BDPLT17
Gene/locus	GFI1B
Method of modification	–
Name of transgene or resistance	–
Inducible/constitutive system	–
Date archived/stock date	December 2015
Cell line repository/bank	–
Ethical approval	Informed consent was given in accordance with the Declaration of Helsinki and Dutch national and Sanquin internal ethic boards

Resource utility

We generated the iPSC line harboring the GFI1B p287* mutation. Besides the conventional application of iPSC, this line can be useful to study the role of GFI1B in various tissue and in particular during megakaryopoiesis.

Resource details

Peripheral blood mononuclear cells (PBMC) were collected from a female individual carrying a heterozygous c.859C → T p.Q287* mutation in growth factor independent 1B (GFI1B). The mutation has been described to cause autosomal dominant gray platelet syndrome (OMIM # 187900, BLEEDING DISORDER, PLATELET-TYPE, 17; BDPLT17) (Monteferrario et al., 2014). The premature stop codon at position 287

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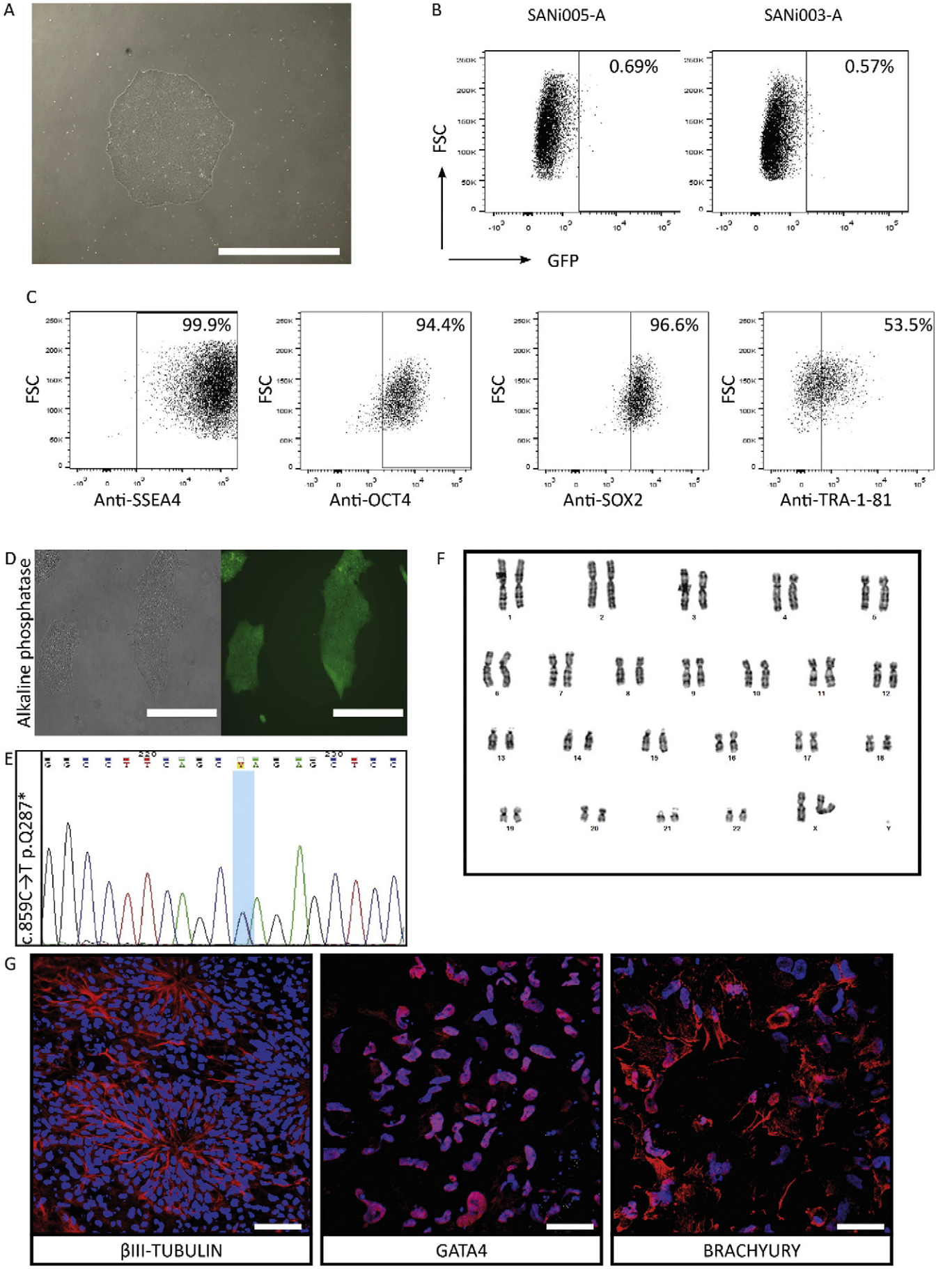

Fig. 1. Characterization of SANi005-A iPSC line.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1A
Phenotype	Immunocytochemisty	Alkaline phosphatase positive	Fig. 1D
	Flow cytometry	OCT/4: 94.4% TRA-1-81: 53.5% SSEA-4: 100% SOX2: 96.6%	Fig. 1C
Genotype	Karyotype (G-banding) and resolution	46XX, Resolution 500	Fig. 1F
Identity	Microsatellite PCR (mPCR) STR analysis	Not performed Performed on iPSC	
Mutation analysis	Sequencing Southern Blot OR WGS	c.859C → T p.Q287* Not performed	Fig. 1E
Microbiology and virology	Mycoplasma	Negative by MycoAlert Mycoplasma detection kit (Lonza # LT07-118)	Not shown but available with author
Differentiation potential	Embryoid body formation	Embryoid body formation to all three germ layers	Fig. 1G
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative	not shown but available with author
Genotype additional info	Blood group genotyping HLA tissue typing	MLPA Unknown	not shown but available with author

results in a truncated protein with an incomplete zinc finger 5 and missing the last zinc finger. The mutant transcript is not targeted by nonsense mRNA mediated decay (Monteferrario et al., 2014). Patients harboring this mutation show aberrant megakaryopoiesis and platelet production/function (Monteferrario et al., 2014). Patient PBMCs were isolated from blood and cultured towards the erythroblast (EBL) lineage as described before (Heideveld et al., 2015). Erythroblast (EBLs) was transduced with the self-inactivating pRRL.PPT.SF.hOKSMco.GFP.preFRT lentiviral vector. Reprogramming was performed on an irradiated mouse embryonic fibroblast (iMEF) feeder layer. The iPSC-like colonies were individually picked 14–20 days post-transduction and SANi005-A iPSC line was chosen for further examination based on morphology criteria (Fig. 1A, scale bare 400 μm) (Tables 1 and 2).

No green fluorescence was detected in the lentivirally reprogrammed SANi005-A iPSC line which was similar to a non-GFP episomal control iPSC line (SANi003-A), indicating silencing of the reprogramming cassette (Fig. 1B). Pluripotency markers SOX2, OCT4, SSEA4 and TRA-1-81 were expressed and iPSC colonies stained positive for alkaline phosphatase (Fig. 1C, D scale bare 400 μm). The disease-causing c.859C → T mutation was confirmed in SANi005-A by Sanger sequencing (Fig. 1E). The genomic integrity of SANI005-A was assessed by Giemsa-banding, indicating normal diploid 46, XX karyotype, without any detectable abnormalities (Fig. 1F). In vitro spontaneous differentiation revealed commitment to the three germline layers (shown in red) by expression of ectodermal (βIII-TUBULIN), endodermal (GATA4) and mesodermal (BRACHYURY) markers (Fig. 1G, blue Dapi, scale bare 40 μm). In conclusion, we have generated iPSC from hematopoietic cells (erythroblasts) of a gray platelet-like BDPLT17 syndrome patient. The generated iPSCs can be used to study BDPLT17 pathophysiology and basic functions of GFI1B. Of note, besides this single fully characterized line we have multiple (>15) additional not fully characterized lines from this patient.

Materials and methods

Experimental procedures

All used chemicals were purchased from Sigma-Aldrich (Munich, Germany) and all culture reagents from Thermo Fisher Scientific (Waltham, Massachusetts, USA), unless otherwise specified.

Cell culture

All cells were cultured at 37 °C in humidified atmosphere containing 5% CO_2. The iPSCs were cultured on Matrigel (BD Biosciences, Breda, The Netherlands) in essential-8 medium (E8) following the manufacturer's instructions.

Isolation of primary cell source and reprogramming of EBLs

PBMC-derived EBLs were cultured as described previously (Monteferrario et al., 2014). Briefly, PBMCs were isolated by ficoll gradient and cultured in expansion medium supplemented with 100 ng/ml SCF (R&D systems, Minneapolis, USA), 1 ng/ml IL-3 (R&D systems), 2 U/ml EPO (R&D systems), and 1 μM Dexamethasone (IL-3 was added till day 2). 5×10^6 EBLs were transduced with pRRL.PPT.SF.hOKSMco.idTomato.preFRT lentivirus (Warlich et al., 2011; Voelkel et al., 2010). 3 days post-transduction the cells were seeded onto irradiated-MEF (GlobalStem, Gaitherburg, USA) in E8 medium supplemented with 100 ng/ml SCF (R&D systems) and 2 mM valproic acid (VPA) (1×10^6 EBLs/ml). From day 5 post-transduction E8

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Differentiation markers	Mouse Neuronal Class III B-TUBULIN	1:2000	Covanc, # MMS-435P, AB_2313773
	Rabbit anti-Brachyury	1:50	Santa Cruz, #sc-20,109, AB_2255702
	Mouse anti-GATA4	1:50	Santa Cruz, #sc-25,310, AB_627667
	Alkaline phosphates	1:500	Thermo Fisher Scientific, #A14353, No RRID
Secondary antibodies	Donkey anti-Mouse IgG (H + L) secondary antibody	1:2000	Thermo Fisher Scientific, A-21202, AB_141607
	Goat anti-Rabbit IgG (H+L) secondary antibody	1:2000	Thermo Fisher Scientific, A-11008, AB_143165
Pluripotency markers flow cytometry	TRA-1-81	1:100	Stem Cell Technologies, # 60065AZ.1, AB_1118559
	OCT4	1:10	R&D Systems, IC1759A-100, AB_1152112
	SOX2	1:10	R&D Systems, IC2018P-100, AB_357273
	SSEA4	1:10	R&D Systems, FAB1435C, AB_1208043

medium + VPA was added without SCF. From day 7 post-transduction the medium was changed to E8 and refreshed every second day hereafter. Day 14–20 post-transduction iPSCs-like colonies were individually isolated and further expanded on Matrigel (BD Biosciences) coated dishes (Table 1).

Karyotyping

Cells were treated with Demecolcine solution (10 μg/ml in HBSS), and processed with standard methods. Giemsa-banded karyotyping was performed and a minimum of 10 metaphases were analyzed. The chromosomes were classified according to the International System for Human Cytogenetic Nomenclature (ISCN, 2016).

In vitro spontaneous differentiation

iPSC cells were harvested using ReLeSR (Stem Cell Technologies, Köln, Germany) according to manufacturer instructions and colony clumps were transferred to an ultra-low attachment dish (Corning, New York, USA) in E8 medium. On day 5 embryonic bodies (EB) were plated on 0.1% gelatin coated coverslips in a 24 wells plate and medium was changed to; 1% Pen/Strep, 20% FBS, 1% 100× MEM Non-essential amino acid solution, 0.1 mM β-mercaptoethanol in DMEM. On day 14 EB were fixed with 4% PFA before staining for all three germ layers (Table 2).

Immunocytochemistry staining

The expression of specific pluripotency and germ layer markers was analyzed using immunocytochemistry staining. The antibodies and applied dilutions are listed in Table 2. Cells were imaged using a LSM510 confocal with META detector (Carl Zeiss) and figures generated with Zen black edition microscope software (Carl Zeiss).

Alkaline phosphatase staining

Alkaline phosphatase staining was performed by alkaline phosphatase live stain (AP) (Thermo Fisher Scientific). In short, the cultured iPSCs were washed with DMEM/F12 prior to staining. E8 was added on plates with AP live stain and incubated for 30 min, then washed 2× with DMEM/F12. Pictures were taken with EVOS-FL (Thermo Fisher Scientific).

Flow cytometry

iPSC single cell suspensions were made using TrypLE Select, and cells stained for pluripotency markers. Anti-TRA-1-81-APC (1:100; Stem cell technologies), OCT4, SOX2, SSEA4 were performed according to manufacturer instructions (R&D Systems). Cells were washed and measured on an LSR-II (BD Bioscience) and analyzed using Flowjo software (Flowjo, Ashland, USA).

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