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Lab resource: Stem Cell Line

Induced pluripotent stem cell line, IMGTi003-A, derived from skin fibroblasts of an intellectually disabled patient with ring chromosome 13

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A B S T R A C T

Skin fibroblasts from a patient with neurodevelopmental and speech delay, anxiety disorder, macrocephaly, microorchidism, multiple anomalies of internal organs and ring chromosome 13 were reprogrammed into induced pluripotent stem cells (iPSCs) to generate a clonal stem cell line IMGTi003-A (iTAF6-6). IMGTi003-A pluripotency was demonstrated by three germ layer differentiation capacity *in vitro*, and this cell line had a mosaic karyotype with 46,XY,r(13) as a predominant cell subpopulation. IMGTi003-A line is a good model for studying of the mitotic instability of the ring chromosome 13.

Resource table

Unique stem cell line identifier	IMGTi003-A
Alternative name(s) of stem cell line	iTAF6–6
Institution	Research Institute of Medical Genetics, Tomsk NRMC, Institute of Cytology and Genetics, SB RAS
Contact information of distributor	Nikitina Tatiana, t.nikitina@medgenetics.ru
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 17 y Sex: Male Ethnicity: Caucasian
Cell Source	Human skin fibroblasts TAF-6
Clonality	Clonal
Method of reprogramming	Lentiviral reprogramming with four transcription factors (OCT4, SOX2, KLF4, C-MYC)
Genetic Modification	No
Type of Modification	n/a
Associated disease	Neurodevelopmental and speech delay
Gene/locus	arr[GRCh38] 3q12.2(100635768_100709492)×4 pat,13q34(112275797_114293545)×1 dn,13q12.11-q34(19207584_114293545)×1 dn
Method of modification	n/a
Name of transgene or resistance	n/a
Inducible/constitutive system	n/a
Date archived/stock date	June 2017
Cell line repository/bank	Bioresource collection of the Research Institute of Medical Genetics, Tomsk NRMC, "Biobank of the population of Northern Eurasia"; Collective Center of ICG SB RAS "Collection of Pluripotent Human and Mammalian Cell Cultures for Biological and Biomedical Research"
Ethical approval	Written informed consent was obtained from the patient's parents. The study was approved by the Scientific Ethics Committee of Research Institute of Medical Genetics, Tomsk NRMC (protocol number 106/2017)

Resource utility

The presence of ring chromosome induces secondary chromosome rearrangements *in vivo* and *in vitro*; the ring chromosome can change in size, become lost or cause derivatives. The IMGTi003-A cell line was generated from a patient with r(13) and represents a well-characterized resource for studying ring chromosome instability in iPSCs.

Resource details

Human skin fibroblasts TAF6 were derived from a 17-year-old male with neurodevelopmental and speech delay, anxiety disorder, macrocephaly, microorchidism, multiple internal organs anomalies and ring

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel C
Phenotype	Qualitative analysis Immunocytochemistry	Assessed presence of pluripotency markers: OCT4, NANOG, SOX2, SSEA4, TRA-1-60	Fig. 1 panel B
	Quantitative analysis Immunocytochemistry counting	OCT4:98.5%; SOX2: 98.6%; NANOG:98.4%; SSEA4:98.8%; TRA-1-60: 98.3%.	Fig. 1 panel B
Genotype	Karyotype (G-banding) and resolution	46,XY,r(13)[19]/45,XY,-13[2]/46,XY,-13, + mar[1] at passage 17; 46,XY,r(13)[32]/47,XY,r13, + mar[17]/46,XY,-13, + mar[1] at passage 20 Resolution 450 band	Fig. 1 panel A
Identity	STR analysis	19/19 sites completely matched	submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	n/a	n/a
	Southern Blot OR WGS	n/a	n/a
Microbiology and virology	Mycoplasma	*Mycoplasma* testing by PCR: Negative	Fig. 1 panel F
Differentiation potential	Embryoid body formation	Expression of genes of the three germ layers in embryoid bodies (*AFP, HNF-3B* for endoderm; *TBXT, MSX1, FLK1* for mesoderm; *SOX1*, *PAX6* and *MAP2* for ectoderm)	Fig. 1 panel E
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	n/a	n/a
Genotype additional info (OPTIONAL)	Blood group genotyping	n/a	n/a
	HLA tissue typing	n/a	n/a

chromosome 13 with *de novo* del13q34, and trip3q12, inherited from apparently healthy father. The karyotype in the patient's blood lymphocytes was 46,XY,r(13)[18] by GTG-analysis, with 39% of monosomy 13 by interphase FISH. The karyotype of the fibroblasts was assessed at different passages and showed “dynamic mosaicism”, typical for cells with ring chromosome. The major karyotype in fibroblasts was 46,XY,r(13) with minor karyotypes 45,XY,-13 and 46,XY,-13, + mar, and sporadically some others karyotypes (data not shown).

Fibroblasts were reprogrammed into iPSCs through lentiviral delivery of four reprogramming factors (OCT4, SOX2, KLF4, and C-MYC) using protocol described by (Gridina et al., 2018) (Table 1). Four pluripotent iPSC lines were generated, but in this study we characterized only IMGTi003-A (iTAF6-6) line.

IMGTi003-A line displayed human embryonic stem cell-like morphology in phase contrast microscopy (Fig. 1C), and expressed OCT4, NANOG and SOX2 pluripotency markers in the nucleus and SSEA4 and TRA-1-60 surface markers, as detected by immunofluorescence staining (Fig. 1B). RT-PCR analysis revealed that IMGTi003-A line expressed the pluripotency marker genes - *SOX2, OCT4* and *NANOG* (Fig. 1D). Immunocytochemistry counting allowed to quantify the percentage of IMGTi003-A cells positive for OCT4, SOX2, NANOG, SSEA4 and TRA-1-60 as 98.5%, 98.6%, 98.4%, 98.8% and 98.3%, respectively (Table 1).

IMGTi003-A cell line, as well as other cell lines obtained in this study, had mosaic karyotype, as determined by G-banding with 400–450 band resolution. Due to mitotic instability of ring chromosomes, 86% of cells in the IMGTi003-A line had 46,XY,r(13) karyotype at passage 17 with 45,XY,-13 and 46,XY,-13, + mar karyotypes as minor classes (9% and 5%, respectively). At passage 20 the proportion of cells with 46,XY,r(13) karyotype decreased to 64%, whereas cells with 47,XY,r(13), + mar karyotype accounted for 34% (Fig. 1A). Therefore, modal classes of karyotypes were present both in fibroblasts and IPSCs and reprogramming seems not to induce karyotype instability in this case.

The STR profile of the IMGTi003-A cell line matched with that of the parental TAF6 fibroblasts (loci analyzed: *D1S1656, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D21S11, D22S1045, AMEL, CSF1PO, FGA, SE33, TH01, TPOX* and *vWA*).

The IMGTi003-A cell line was negative for *Mycoplasma* contamination (Fig. 1F).

The ability of the IMGTi003-A cell line to differentiate into cells of the three germ layers following embryoid body formation was assessed by qPCR for the endodermal (*AFP, HNF-3B*), mesodermal (*TBXT (BRACHYURY), MSX1, FLK1*) and ectodermal (*SOX1*, *PAX6* and *MAP2*) genes (Fig. 1E). These results demonstrated that the IMGTi003-A cells are pluripotent.

Materials and methods

Cell culture

TAF6 human fibroblasts were derived from a patient carrying r(13) and cultured in growth media (DMEM/F12 (Gibco) supplemented with 10% fetal bovine serum (FBS) (HyClone), 1% Pen Strep (Gibco), 1% MEM Non-essential Amino Acid solution, 1% MEM Vitamin solution, 2 mM L-glutamine (all from Sigma)) at 37 °C in 5% CO_2.

Generation of iPSCs from r(13) patient fibroblasts

To produce iPSC from the patient's fibroblasts we used modified LeGO-G2 lentiviral vectors (Addgene, #25917) with eGFP substituted by the human reprogramming transcription factors OCT4, SOX2, C-MYC, and KLF4. These vectors were kindly provided to us by Dr. Sergei L. Kiselev (Moscow, Russia). The lentiviruses were produced in the Phoenix cell line using Lipofectamine 3000 (Invitrogen) according to the manufacturer's recommendations. Fibroblasts plated on the previous day were transduced with viruses containing the four reprogramming transcription factors for two days (on the second day, the C-MYC lentivirus was omitted). From day 6 to 16 the culture medium was changed daily with addition of 1 mM valproic acid (Sigma). On day 5, the transduced cells were seeded onto 10-cm culture dishes (2×10^3 cells per cm^2) containing mitomycin C-treated CD-1 mouse embryonic fibroblast feeder cells in iPSC medium (DMEM/F12 medium supplemented with 20% KnockOut Serum Replacement, 1% GlutaMAX™-I, 1% MEM NEAA, 1% Pen Strep (all from Gibco), 0.1 mM 2-mercaptoethanol, and 10 ng/ml bFGF (Invitrogen)). On day 18, colonies with iPSC morphology were picked up and expanded. All cell cultures were maintained at 37 °C in an atmosphere of 5% CO_2. iPSCs maintained on feeder cells were expanded mechanically or by enzymatic dissociation using TripLE (Gibco).

Immunocytochemistry

The iPSCs fixed with 3% formaldehyde for 20 min at room

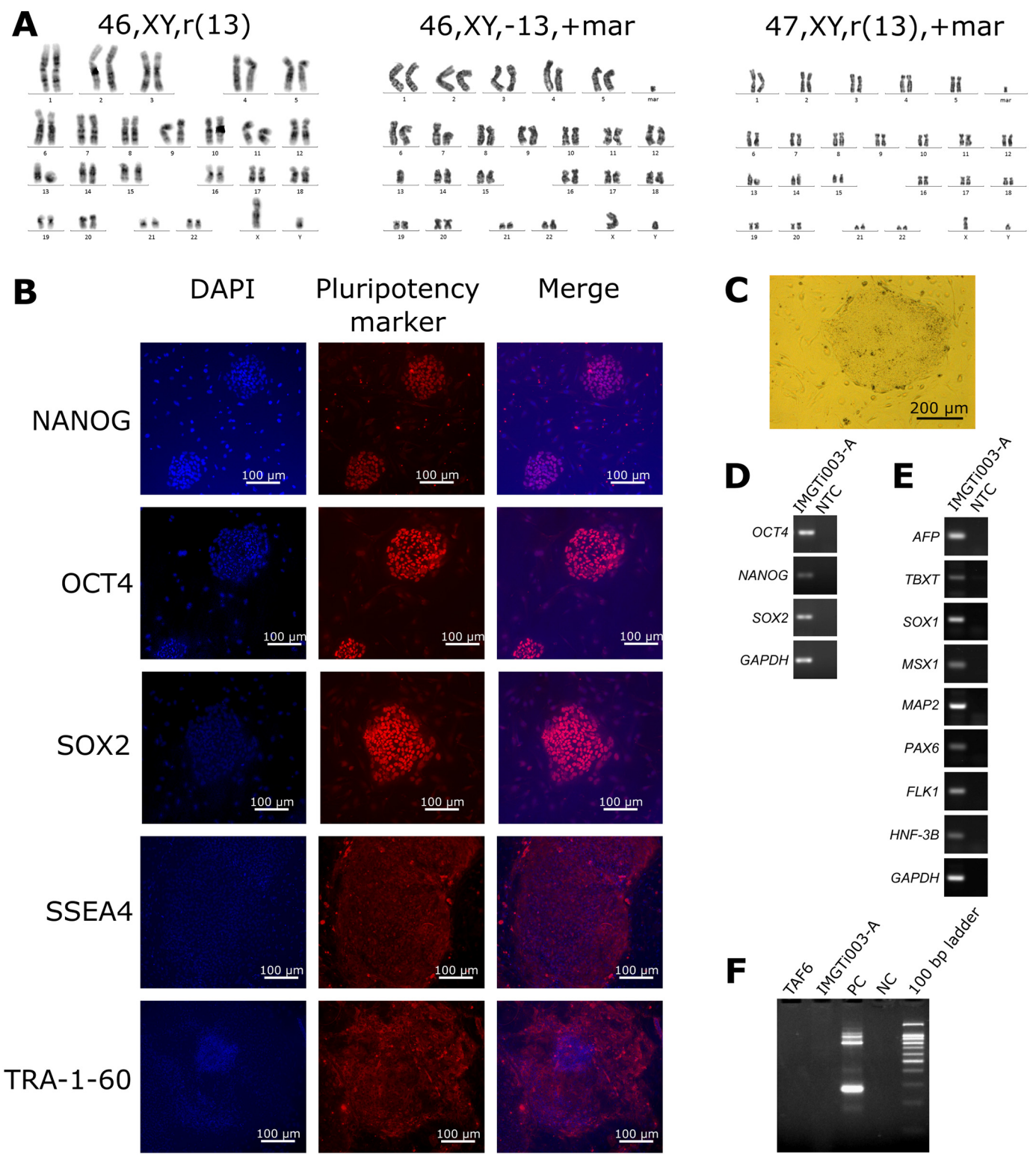

Fig. 1. Characterization of IMGTi003-A iPSC line. (A) Karyotypes. (B) Immunofluorescence staining for the pluripotency markers NANOG, OCT4, SOX2, SSEA4, TRA-1-60. (C) Morphology of the iPSC colonies. (D) Expression of the pluripotency markers OCT4, NANOG, SOX2. (E) Expression of the endoderm (AFP, HNF-3B), mesoderm (TBXT, MSX1, FLK1) and ectoderm (SOX1, PAX6, MAP2) markers in the embryoid bodies. (F) *Mycoplasma* contamination test.

temperature. After fixation, the cells were permeabilized with 0.1% Triton X-100 in PBS for 3 min at room temperature, blocked with 3% bovine serum albumin in PBS for 25 min and incubated overnight at 4 °C with primary antibodies. Secondary antibodies were incubated for 1 h at room temperature (Table 2) and further counterstained with DAPI. Immunofluorescence was examined with a fluorescence microscope AxioObserver Z1 (Zeiss) using ZEN software in collective Microscopic Center of ICG SB RAS, Novosibirsk.

In vitro differentiation

Embryoid bodies were produced according to previously published

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-NANOG	1:100	Abcam Cat# 21624, RRID: AB_446437
Pluripotency Markers	Rabbit anti-OCT4	1:200	Abcam Cat# 19857, RRID: AB_445175
Pluripotency Markers	Mouse anti-SSEA4	1:600	Abcam Cat# 16287, RRID:AB_778073
Pluripotency Markers	Mouse anti-TRA-1-60	1:600	Abcam Cat# 16288, RRID:AB_778563
Pluripotency Markers	Mouse anti -SOX2	1:400	RSE National center for Biotechnology, Astana, Cat# NCB 1601
Secondary antibodies	Alexa Fluor 546 anti-Mouse IgG	1:500	Thermo Fisher Scientific Cat# A-11060, RRID:AB_2534107
Secondary antibodies	Alexa Fluor 546 Goat Anti- Rabbit IgG	1:500	Life technologies Cat# A-11010, RRID:AB_143156

Primers		
	Target	Forward/Reverse primer (5′-3′)
Pluripotency Marker	*OCT4*	CTGGGTTGATCCTCGGACCT/CACAGAACTCATACGGCGGG
Pluripotency Marker	*NANOG*	AAAGAATCTTCACCTATGCC/GAAGGAAGAGGAGAGACAGT
Pluripotency Marker	*SOX2*	GCATCGCAGCTTGGATACAC/GCTTCAGCTCCGTCTCCAT
House-Keeping Gene	*GAPDH*	GTGGACCTGACCTGCCGTCT/GGAGGAGTGGGTGTCGCTGT
Differentiation Marker	*AFP*	AAATGCGTTTCTCGTTGCTT/GCCACAGGCCAATAGTTTGT
Differentiation Marker	*TBXT* (*BRACHYURY*)	AATTGGTCCAGCCTTGGAAT/CGTTGCTCACAGACCACA
Differentiation Marker	*SOX1*	CACAACTCGGAGATCAGCAA/GGTACTTGTAATCCGGGTGC
Differentiation Marker	*PAX6*	GTCCATCTTTGCTTGGGAAA/TAGCCAGGTTGCGAAGAACT
Differentiation Marker	*MAP2*	CAGGTGGCGGACGTGTGAAAATTGAGAGTG/CACGCTGGATCTGCCTGGGGACTGTG
Differentiation Marker	*HNF-3B*	GGAGCGGTGAAGATGGAA/ TACGTGTTCATGCCGTTCAT
Differentiation Marker	*MSX1*	CGAGAGGACCCCGTGGATGCAGAG/ GGCGGCCATCTTCAGCTTCTCCAG
Differentiation Marker	*FLK1*	TGATCGGAAATGACACTGGA/ CACGACTCCATGTTGGTCAC

protocol (Bock et al., 2011).

Karyotyping

Preparation of metaphase chromosomes from iPS cells was performed as previously described with minor modifications (Prokhorovich et al., 2007). Karyotype analysis was performed using conventional GTG banding techniques according to standard cytogenetic protocols based on the International System for Human Cytogenomic Nomenclature (2016). As far as IMGTi003-A line is mosaics we have analyzed from 20 to 50 metaphases for sample at different passages (450-band resolution).

Immunocytochemistry counting

To quantify the percentage of IMGTi003-A cells positive for OCT4, SOX2, NANOG, SSEA4 and TRA-1-60 we used software ImageJ (platform Fiji) and determined the signal as positive if the fluorescence intensity was more than two times higher than that of the negative control (Schindelin et al., 2012).

Mycoplasma contamination detection

The absence of *Mycoplasma* contamination was confirmed by PCR using primers from Choppa et al., 1998.

STR analysis

Parent TAF6 fibroblasts and their derivative IMGTi003-A (iTAF6-6) iPSC line were authenticated by STR analysis by Gordiz (http://gordiz.ru/).

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