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Lab Resource: Multiple Cell Lines

Generation of two iPSC lines (IMGTi001-A and IMGTi001-B) from human skin fibroblasts with ring chromosome 22

Nikitina T.V.[a,*], Menzorov A.G.[b,c], Kashevarova A.A.[a], Gridina M.M.[b], Khabarova A.A.[b], Yakovleva Yu.S.[a], Lopatkina M.E.[a], Kizilova E.A.[b], Vasilyev S.A.[a], Serov O.L.[b,c], Lebedev I.N.[a]

[a] Research Institute of Medical Genetics, Tomsk NRMC, Tomsk, Russia
[b] Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia
[c] Novosibirsk State University, Novosibirsk, Russia

A B S T R A C T

Skin fibroblasts from a patient with intellectual disability and ring chromosome 22 were reprogrammed into induced pluripotent stem cells (iPSCs) to establish a clonal stem cell lines, IMGTi001-A (iTAF5-29) and IMGTi001-B (iTAF5-32). Because of ring chromosome mitotic instability these cell lines show mosaic karyotypes with 46,XX,r(22) in > 83% cells, 45,XX,-22 as minor class and sporadically cells with other karyotypes. Differentiation in derivatives of all three germ layers was shown in teratoma assay for IMGTi001-A, and in embryoid bodies for both cell lines. To our knowledge, human iPSC lines with ring chromosome are described for the first time.

Resource table

Unique stem cell lines identifier	IMGTi001-A IMGTi001-B
Alternative names of stem cell lines	iTAF5-29 iTAF5-32
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 lines	iPSC
Origin	Species: Human Age: 4 y Sex: Female
Cell source	Human skin fibroblasts TAF5
Clonality	Clonal
Method of reprogramming	Lentiviral reprogramming with four transcription factors (OCT4, SOX2, KLF4, C-MYC)
Multiline rationale	Isogenic cell lines
Gene modification	No
Type of modification	n/a
Associated disease	Phelan-McDermid syndrome (OMIM 606232), 3q13.31 deletion syndrome (OMIM 615433)
Gene/locus	46,XX,r(22), arr[hg19] 3q13.31(116233164_116615500) × 1,22q13.32(48886812_49059015) × 3,22q13.32-q13.33(49115584_51178264) × 1

* Corresponding author.
E-mail address: t.nikitina@medgenetics.ru (T.V. Nikitina).

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Method of modification	n/a
Name of transgene or resistance	n/a
Inducible/ constitutive system	n/a
Date archived/stock date	December 2016 (IMGTi001-A) July 2017 (IMGTi001-B)
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

It is assumed that mitotic inheritance of ring chromosomes in iPSCs is very unstable, and ring chromosomes are incompatible with pluripotency (Bershteyn et al., 2014). The established iPSC lines (IMGTi001-A and IMGTi001-B) provide a source to study ring chromosomes stability in iPSCs.

Resource details

Human skin fibroblast (HSF) cells TAF5 from a patient with intellectual disability and ring chromosome 22 (Kashevarova et al., 2018) 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). The established IMGTi001-A and IMGTi001-B lines had human embryonic stem cell-like morphology in phase contrast microscopy (Fig. 1D), and expressed OCT4, NANOG and SOX2 pluripotency markers in the nucleus and SSEA4, TRA-1-60 and TRA-1-81surface markers, as detected by immunofluorescence staining (Fig. 1B, Supplementary Fig. 1A). RT-PCR analysis revealed that both iPSC lines expressed pluripotency marker genes *SOX2, OCT4,* and *NANOG* (Fig. 1E).

Immunocytochemistry counting revealed the percentage of cell positive for OCT4 (> 97%), SOX2 (> 96%), NANOG (> 95%), SSEA4 (> 98%) and TRA-1-60 (> 97%) for IMGTi001-A and IMGTi001-B.

Both iPSC lines had mosaic karyotypes with 46,XX,r(22) in 83% cells in line IMGTi001-A at passage 23 and 86% cells in line IMGTi001-B at passage 17, with 45,XX,-22 as minor class and sporadically cells with other karyotypes, as determined by G-banding with 400–450 band resolution (Fig. 1A). The STR profiles of cell lines matched 100% with that of the parental.

fibroblast cells TAF5 (loci analyzed: D1S1656, D2S441, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D21S11, D22S1045, AMEL, CSF1PO, FGA, SE33, TH01, TPOX, and vWA). All the cell lines were negative for Mycoplasma contamination (Supplementary Fig. 1B).

To test the differentiation potential of IMGTi001-A and IMGTi001-B, we performed differentiation assays both *in vitro* and *in vivo*. The embryoid body formation assay showed that the lines could spontaneously differentiate into cells that expressed AFP (endoderm), TBXT (BRACHYURY) (mesoderm) and SOX1, PAX6 and MAP2 (ectoderm) (Fig. 1F). Haematoxylin-eosin staining of tumor samples generated from the cell line IMGTi001-A revealed non-squamouos ectodermal epithelium in ectopic site (left); gut-like structure in ectopic site, villi and cryptae covered by enzyme-secreting entodermal epithelium (middle); chondroid or cartilage lense in ectopic site (right) (Fig. 1C). These results demonstrated that IMGTi001-A and IMGTi001-B cells are pluripotent and could differentiate into all three germ layers.

Materials and methods

Cell culture

Human fibroblasts TAF5 were derived from patient carrying r(22) 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(22) patient fibroblasts

To produce iPSC from the patient's fibroblasts we used LeGO lentiviral vectors containing the human reprogramming transcription factors OCT4, SOX2, C-MYC, and KLF4. The lentiviruses were produced in the Phoenix cell line using Lipofectamine 3000 (Invitrogen) according to the manufacturer's recommendations. The LeGO lentiviral vectors containing the human reprogramming transcription factors OCT4, SOX2, C-MYC, and KLF4 were kindly provided to us by Dr. Sergei L. Kiselev (Moscow, Russia). 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). Until day 10, 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 (MEF) feeder cells in iPS cell 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/

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
IMGTi001-A	IMGTi001-A	Female	3	Caucasian	r(22), del22q13.32-q13.33, dup22q13.32, del3q13.31	Phelan-McDermid syndrome, 3q13.31 deletion syndrome
IMGTi001-B	IMGTi001-B	Female	3	Caucasian	r(22), del22q13.32-q13.33, dup22q13.32, del3q13.31	Phelan-McDermid syndrome, 3q13.31 deletion syndrome

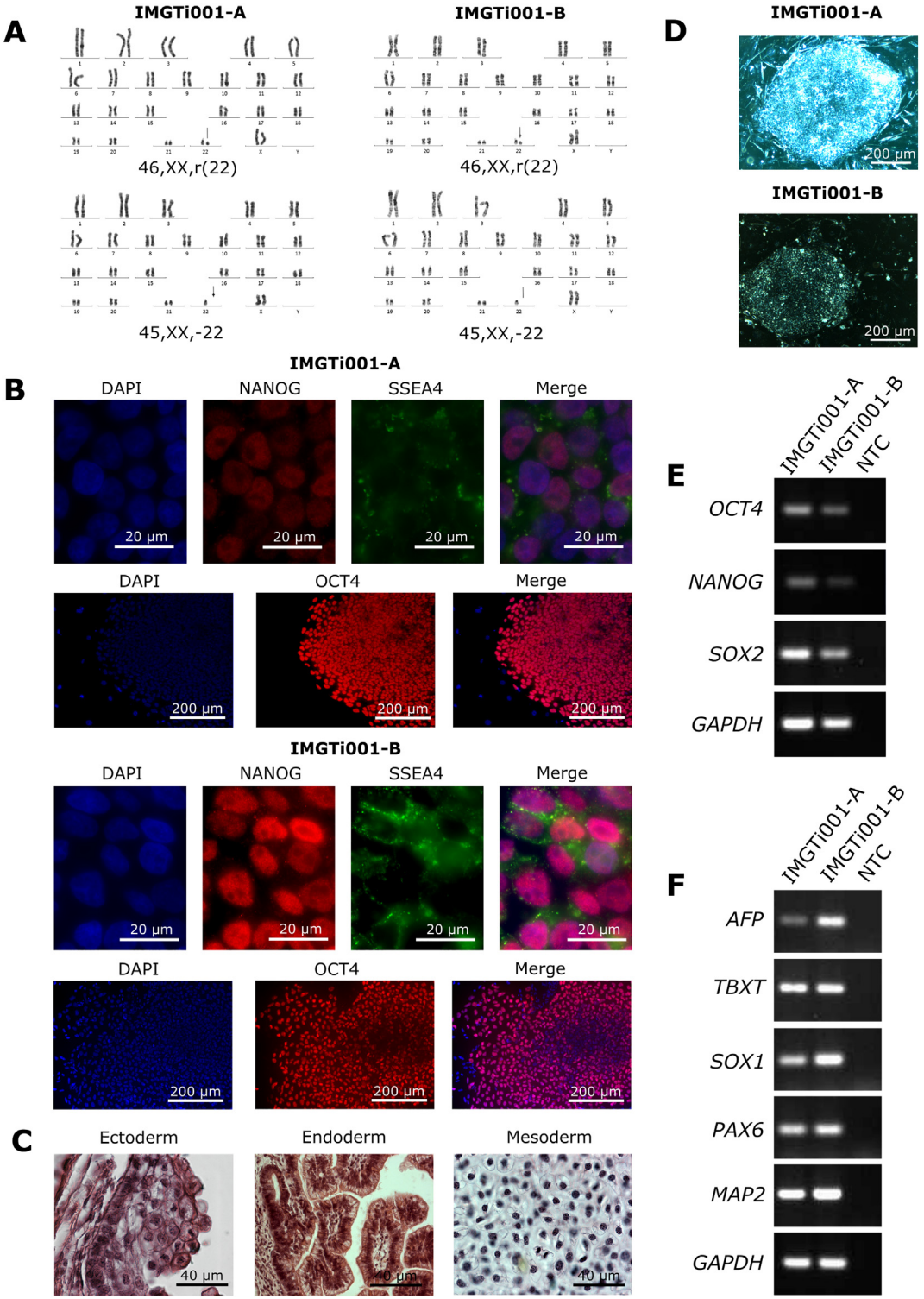

Fig. 1. Characterization of the iPSC lines IMGTi001-A and IMGTi001-B. (A) Karyotypes. (B) Immunofluorescence staining for the pluripotency markers NANOG, SSEA4, OCT4. (C) Teratoma assay. (D) Morphology of the iPSC colonies. (E) Expression of the pluripotency markers OCT4, NANOG, SOX2. (F) Expression of the endoderm (AFP), mesoderm (TBXT) and ectoderm (SOX1, PAX6, MAP2) markers.

ml bFGF (Invitrogen)). On day 18, colonies with iPS cell morphology were picked and expanded. All cell cultures were maintained at 37 °C in an atmosphere of 5% CO2. IPSCs maintained on MEFs were expanded mechanically or by enzymatic dissociation using TripLE (Gibco) (Table 2).

Immunocytochemistry

The iPSC were fixed in 3% paraformaldehyde for 20 min at 4 °C, blocking of unspecific sites was achieved by incubation with 5% FBS in the presence of 0.1% Triton-X100 for 20 min at room temperature. Primary antibodies were incubated overnight at 4 °C, while secondary antibodies were incubated for 1 h at room temperature (Table 3).

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel D
Phenotype	Qualitative analysis immunocytochemistry	Assess staining of pluripotency markers: OCT4, NANOG, SOX2, SSEA4, TRA-1-60, TRA-1-81	Fig. 1 panel B Supplementary Fig. 1 panel A
	Quantitative analysis immunocytochemistry counting	IMGTi001-A: OCT4:97.4%; SOX2: 96.2%; NANOG:98.6%; SSEA4:98.7%; TRA-1-60: 97.6%. IMGTi001-B: OCT4:98.2% SOX2:97.22% NANOG: 95.24% SSEA4:98.8% TRA-1-60:97.9%	Fig. 1 panel B
Genotype	Karyotype (G-banding) and resolution	IMGTi001-A 46,XX,r(22)[41]/45,XX,-22[9] IMGTi001-B 46,XX,r(22)[43]/45,XX,-22[7] Resolution 450	Fig. 1 panel A
Identity	STR analysis	20/20 sites 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	Supplementary Fig. 1 panel B
Differentiation potential	Embryoid body formation, teratoma formation for IMGTi001-A only	Expression of genes of the three germ layers in embryoid bodies (AFP for endoderm; TBXT (BRACHYURY) for mesoderm; SOX1, PAX6 and MAP2 for ectoderm); teratoma sections shows ectoderm-, endoderm- and mesoderm-like structures	Fig. 1 panel C and F
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

Table 3
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-TRA-1-81	1:600	Abcam Cat# 16289, RRID:AB_2165986
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
Secondary antibodies	Alexa Fluor 488 Goat Anti-Mouse IgG	1:500	Life technologies Cat# A-11029, RRID:AB_138404

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

Nuclei were stained with VECTASHIELD® with DAPI (Vector Laboratories) and immunofluorescence was visualized under fluorescence microscope AxioImager (Zeiss) with the ISIS software (MetaSystems).

In vitro differentiation

Embryoid bodies were produced according to previously published protocol (Bock et al., 2011).

Teratoma formation assay

Teratoma formation assay was carried out for IMGTi001-A line only. The protocol was described by (Gridina et al., 2018).

Karyotyping

Karyotype analysis was performed using conventional GTG banding techniques according to standard cytogenetic protocols based on the International System for Human Cytogenetic Nomenclature (2016). As far as iPSC clones are mosaics we have analyzed at least 50 metaphases for each sample (450-band resolution).

Mycoplasma contamination detection

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

STR analysis

Parent fibroblasts TAF5 and their derivative iPSC lines IMGTi001-A and IMGTi001-B were authenticated by STR analysis by Gordiz (http://gordiz.ru/).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.08.012.

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