Stem Cell Research 41 (2019) 101596

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Multiple Cell Lines

Generation of induced Pluripotent Stem Cells (UNIBSi008-A, UNIBSi008-B, UNIBSi008-C) from an Ataxia-Telangiectasia (AT) patient carrying a novel homozygous deletion in *ATM* gene

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A B S T R A C T

Using a Sendai Virus based vector delivering Yamanaka Factors, we generated induced Pluripotent Stem Cells (iPSCs) from peripheral blood mononuclear cells of a patient affected by Ataxia Telangiectasia (AT), caused by a novel homozygous deletion in *ATM*, spanning exons 5–7. Three clones were fully characterized for pluripotency and capability to differentiate. These clones preserved the causative mutation of parental cells and genomic stability over time (>100 passages). Furthermore, in AT derived iPSCs we confirmed the impaired DNA damage response after ionizing radiation. All these data underline potential usefulness of our clones as *in vitro* AT disease model.

Resource table

Unique stem cell lines identifier	UNIBSi008-A UNIBSi008-B UNIBSi008-C
Alternative names of stem cell lines	AT-MC-C1 (UNIBSi008-A) AT-MC-C2 (UNIBSi008-B) AT-MC-C12 (UNIBSi008-C)
Institution	"Angelo Nocivelli" Institute for Molecular Medicine, Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy
Contact information of distributor	Stefania Masneri: s.masneri003@unibs.it
Type of cell lines	iPSC
Origin	Human
Additional origin info	Age: 19 Sex: female Ethnicity: Caucasian
Clonality	Clonal
Method of reprogramming	CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo-Fisher Scientific). This non-integrating episomal vector system is based on the Yamanaka factors *Oct4, Sox2, Klf4*, and *c-Myc*
Multiline rationale	Isogenic clones
Gene modification	Yes
Type of modification	Hereditary
Associated disease	Ataxia-Telangiectasia (AT)
Gene/locus	ATM: NM_000051
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	April 2019
Cell line repository/bank	https://hpscreg.eu/cell-line/UNIBSi008-A https://hpscreg.eu/cell-line/UNIBSi008-B https://hpscreg.eu/cell-line/UNIBSi008-C
Ethical approval	The study was approved by the Scientific Committee and by the Board of the ASST Spedali Civili of Brescia (NP 3426 – Studio iPSCREP).

1. Resource utility

We generated three AT-iPSC lines, derived from peripheral blood mononuclear cells of a patient carrying a homozygous deletion in *ATM* (spanning exons 5–7). These clones and specific derived cell lines are useful tools for disease modelling, gene therapy, and screening of compounds with therapeutic effect.

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https://doi.org/10.1016/j.scr.2019.101596
Received 26 August 2019; Received in revised form 17 September 2019; Accepted 19 September 2019
Available online 18 October 2019

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
UNIBSi008-A	AT-C1	Female	19	Caucasian	g.[108,105,741–108,115,748 del];[108,105,741–108,115,748 del]	AT
UNIBSi008-B	AT-C2	Female	19	Caucasian	g.[108,105,741–108,115,748 del];[108,105,741–108,115,748 del]	AT
UNIBSi008-C	AT-C12	Female	19	Caucasian	g.[108,105,741–108,115,748 del];[108,105,741–108,115,748 del]	AT

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis (Immunocytochemistry)	Positive for pluripotency markers: OCT4, and TRA–1–60	Fig. 1 panel D
	Quantitative analysis (TaqMan hPSC Scorecard Panel)	All samples were found to be pluripotent and transgene free: positive score for self-renewal gene expression, negative score for ectodermal, mesodermal, and endodermal gene expression.	Fig. 1 panel C
	Qualitative analysis (Functional assessment)	Measure the impaired DNA damage response after ionizing radiation (staining with pH2A.X and pATM)	Fig. 1 panel F
Genotype	Karyotype (Q-banding) and resolution	46,XX Resolution 450–500	Supplementary Fig. 2 (UNIBSi008-A), Supplementary Fig. 3 (UNIBSi008-B), Supplementary Fig. 4 (UNIBSi008-C)
Identity	Microsatellite PCR (mPCR)	N/A	N/A
	STR analysis	16 distinct loci: all matched to parental cell line	Available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	Homozygous g. 108,105,741–108,115,748 del	Fig. 1 panel B
	Southern Blot OR WGS	MLPA assay	Available with the authors
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR: Negative	Supplementary Fig. 1
Differentiation potential	Trilineage Differentiation	The cell lines can differentiate into three germ layers (Ectoderm: *PAX6, SOX1*; Mesoderm: CXCR4, ACTA2; Endoderm GATA4, SOX17)	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

2. Resource details

Ataxia Telangiectasia (AT) is a rare autosomal recessive disorder caused by mutations in the *ATM* gene, coding ATM kinase, a master regulator of the DNA damage response (DDR) after double strand breaks (Corti et al., 2019). AT patients are characterized by a progressive neurological impairment due to cerebellar atrophy, oculocutaneous telangiectasia, predisposition to cancer, immunodeficiency, and, at a cellular level, radiosensitivity, genomic instability, and cell cycle defects (Corti et al., 2019; Chaudhary and Al-Baradie, 2014). To date, no curative therapies are available for AT. Therefore, to better understand the pathophysiology of this disease, we selected a nineteen years old female patient affected by typical AT, in which we identified a large homozygous deletion in *ATM* (g. 108,105,741–108,115,748 del, spanning exons 5–7), using a NGS target sequencing and an MLPA assay (Multiplex Ligation-dependent Probe Amplification, SALSA MLPA P041 ATM-1 probemix, MRC—Holland), and confirmed through Sanger Sequencing.

Using a modified Sendai Virus based vector, carrying the Yamanaka factors *OCT4, SOX2, KLF4,* and *c-MYC,* (CytoTune-iPS 2.0 Sendai Reprogramming Kit, Thermo-Fisher Scientific), we generated induced Pluripotent Stem Cells (iPSCs) in feeder free condition from patient's peripheral blood mononuclear cells (PBMCs). Eight clones were stabilized, and three were selected for a deeper characterization (UNIBSi008-A, UNIBSi008-B, UNIBSi008-C, summarized in Tables 1 and 2).

All clones were mycoplasma-free (Supplementary Fig. 1), displayed ESC-like morphology (Fig. 1A), and retained the parental cells causative mutation (Fig. 1B and Table 3). Unlikely primary AT blood cells, that exhibited chromosomal abnormalities typical of the disease [for example 46,XX,inv(7), 46,XX,del(14q), 46,XX,t(7p;14q)], a standard QFQ-banding of all clones revealed a 46,XX karyotype, normal both in number and structure, which was maintained over time (>100 passages in culture; UNIBSi008-A was karyotyped at passage 14, 36, 67, and 119; UNIBSi008-B at passage 15, 46, 80, and 105; UNIBSi008-C at passage 15, 35, 72, and 128). Representative images are shown in Supplementary Fig. 2, Fig. 3, Fig. 4, respectively. Moreover, STR analysis of 16 loci revealed 100% match between iPSCs and parental cells (AmpFISTR Identifiler Plus (Life Technologies) (available with authors).

TaqMan hPSC Scorecard Panel (Thermo-Fisher Scientific), that evaluates pluripotency and trilineage differentiation potential by a real-time quantitative PCR (qPCR) assays, demonstrated that all samples were pluripotent and transgene-free (Fig. 1C). Pluripotency was also confirmed by immunofluorescence staining of two stem cell markers: Tra-1-60, expressed on cell surface, and OCT4, present at nuclear level (Fig. 1D), using the antibodies itemized in Table 3.

Differentiation ability of all clones were tested through *in vitro* spontaneous differentiation in the three germ layers and the gene expression analysis of ectodermal, mesodermal and endodermal markers (*PAX6-SOX1, CXCR4-ACTA2, GATA4-SOX17*, respectively) (Table 3 and Fig. 1E).

Finally, we developed a functional assay based on flow cytometry to test the radio-sensitivity of AT (PBMCs or iPSCs) samples. Compared to control cells, AT-iPSC clones, as well as AT-PBMCs, showed a reduction in H2A.X and ATM phosphorylation, after DNA damage induced by Ionizing Radiation (IR) (Fig. 1F). The reduced ability to activate DDR is a typical feature of AT cells, maintained also in the iPSCs.

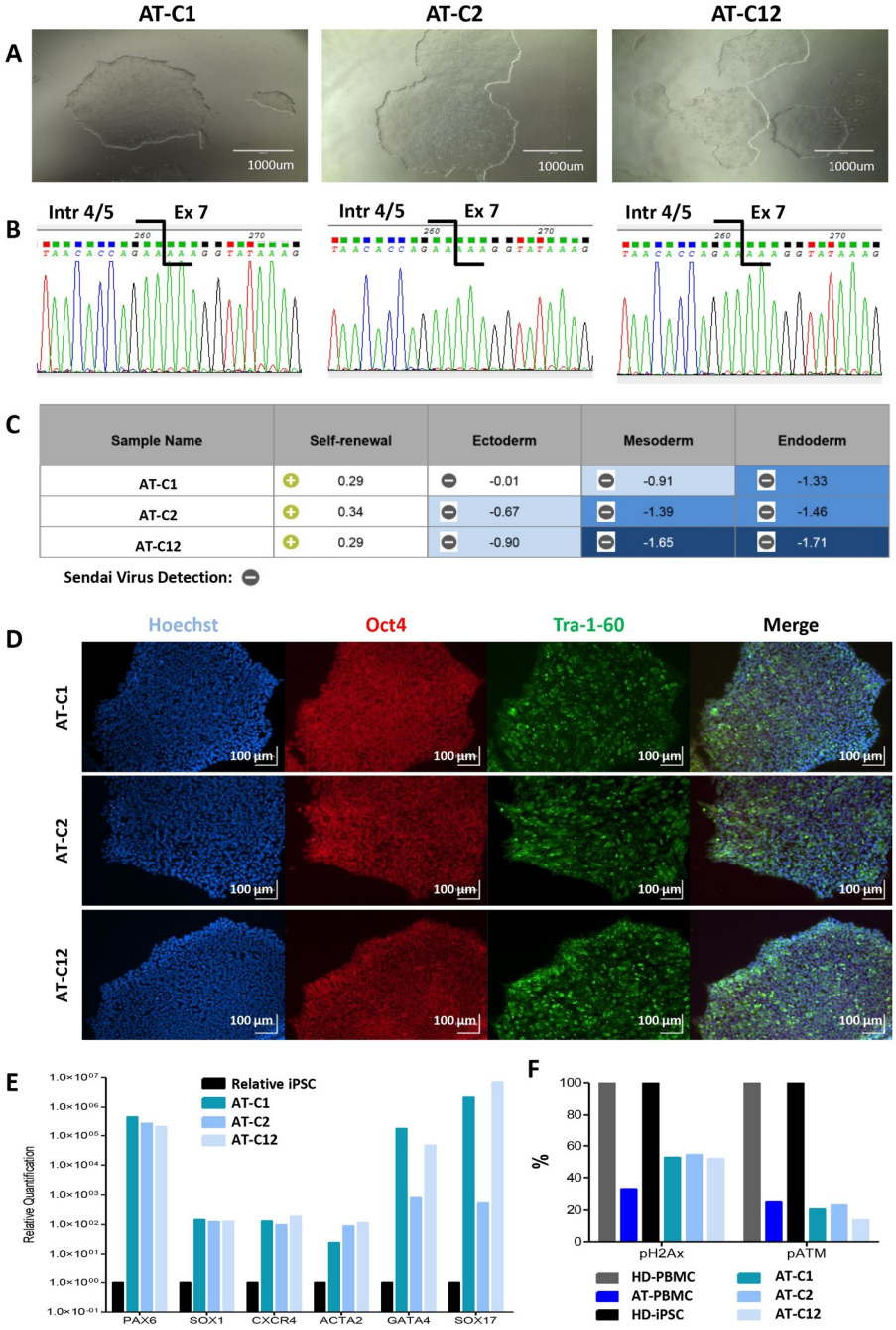

Fig. 1. A. iPSC clones morphology; scale bar = 1 mm. B. iPSCs genomic sequence, showing the junction between intron 4/5 and exon 7, marked with a broken black line. C. TaqMan hPSC Scorecard Report, displaying iPSCs pluripotency, using numerical values and the "Plus" "Minus" graphical symbols. D. Immunofluorescence analysis of pluripotency markers Tra-1-60 (green), and Oct4 (red). Cell nuclei were stained with Hoechst 33342 (blue), and the three channels were merged; scale bar = 100 µm. E. Gene expression analysis of three germ layers markers. F. Radio-sensitivity assay, showing a reduction in H2A.X and ATM phosphorylation in patient specific (AT) PBMCs and iPSCs compared to healthy donor (HD) cells, after DNA damage.

In conclusion, we demonstrated the feasibility of generating iPSCs from AT-PBMCs for the first time, but also the usefulness and applicability of our AT clones for modeling *in vitro* the AT disease, using the radio-sensitivity test.

3. Materials and methods

3.1. Reprogramming of PBMCs

PBMCs were isolated from peripheral blood using Lympholyte-H Cell Separation Media (Cedarlane). Isolated PBMCs were cultured in Stem Pro-34 SFM Medium (Thermo-Fisher Scientific) supplemented with SCF, FLT-3 (100 ng/ml), IL-3 and IL-6 (20 ng/ml) for four days (Gibco). Then, PBMCs were reprogrammed using the CytoTune-iPS 2.0 Reprogramming kit (Thermo-Fisher Scientific), according to the manufacturer protocol. From day 20, emerging colonies were manually picked based on their morphology and Tra-1-60 positivity, maintained and passaged as iPSCs thereafter. iPSCs were cultured on Matrigel-coated plates (Corning) at 37 °C in 5% CO_2, feeding daily with Nutristem hPSC-XF Medium (Biological-Industries), and passaged manually every four-five days, with a 1:2 split ratio.

3.2. Karyotyping

Cytogenetic analysis was executed using conventional Q-banding at 450–500 bands resolution, according to the International System for Human Cytogenetic Nomenclature (2016). A minimum of 20 metaphase spreads were evaluated.

3.3. Mutation analysis

In iPSCs, confirmation of the causative mutation was established by

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat# and RRID
Pluripotency markers	Rabbit anti-OCT4	1:400	Thermo Fisher Scientific, Cat# A-13998. RRID: AB_2534182
Pluripotency markers	Mouse anti–TRA–1–60	1:100	Thermo Fisher Scientific, Cat# 4110000. RRID: AB_2533494
Secondary antibodies	Goat anti rabbit IgG (*H + L*) Alexa Fluor 568	1:300	Thermo Fisher Scientific, Cat# A-11011. RRID: AB_143157
Secondary antibodies	Goat anti mouse IgG (*H + L*) Alexa Fluor 488	1:300	Thermo Fisher Scientific, Cat# A-11001. RRID: AB_2534069
Functional markers	Mouse anti-phospho-Histone H2A.X (Ser139), clone JBW301, FITC conjugate	1:400	Millipore Cat# 16–202A, RRID: AB_568825
Functional markers	Mouse anti-phospho-ATM (Ser1981), clone 10H11.E12 PE conjugate	1:100	Millipore Cat# FCMAB110P, RRID: AB_10562803

Differentiation RT-qPCR assays with TaqMan chemistry		
	Target	Probe
Ectoderm	*PAX6*	Hs.PT.58.25914558
	SOX1	Hs.PT.58.28041414.g
Mesoderm	*ACTA2*	Hs.PT.56a.2542642
	CXCR4	Hs00607978_s1
Endoderm	*GATA4*	Hs.PT.58.259457
	SOX17	Hs.PT.58.24876513
Housekeeping gene	*ACTB*	Hs.PT.39a.22214847

Primers		
	Target	Forward/Reverse primer (5′ – 3′)
Mycoplasma detection	*16S rRNA*	GGGAGCAAACAGGATTAGATACCCT/ TGCACCATCTGTCACTCTGTTAACCTC (268 bp)
Targeted mutation analysis	*ATM* (intr4-5F/intr7-8R)	TTGAGCCTGAGAGGTTGAGG/CCAGTCAACTTAAAATGCTGAC (expected 1768 bp)
Sequencing	*ATM* (7R)	ACAGAATCTGCTACCACTGCT

amplifying and sequencing gDNA with specific primers (Table 3).

3.4. In vitro trilineage differentiation

The iPSCs were plated and maintained for differentiation according to the StemMACS Trilineage Differentiation Kit protocol (Miltenyi Biotec). On day 7, cells were collected for RNA extraction.

3.5. RNA extraction and qPCR for stemness and pluripotency

Total RNA was extracted using NucleoSpin RNA II kit (Macherey-Nagel), and retro-transcribed by ImProm-II Reverse Transcription System (Promega) according to the protocols. RNAs were sent to CellModel Services for the assessment of pluripotency, through the TaqMan hPSC Scorecard Panel (Thermo-Fisher Scientific). Instead, three layers differentiation was tested by qPCR, using iQ MPLX powermix and TaqMan probes (IDT-Integrated DNA Technologies, listed in Table 3) on CFX96 C1000 Touch Real-Time PCR Detection System (Bio-Rad). The relative quantification of target genes was calculated by the $2^{\hat{}-\Delta\Delta Ct}$ method, using *ßACTIN* as housekeeping gene.

3.6. Immunofluorescence staining for three layers differentiation markers

Cells were fixed and permeabilized (Fix&Perm-Reagent, SIC) for 15 min at room temperature (RT). Then a blocking solution iBind Buffer (Invitrogen) was added for 45 min. Afterwards, cells were incubated with primary antibodies for 3 h, washed and then incubated with secondary antibodies for 1 h at RT (Table 3). Nuclei were stained with Hoechst 33342 (Thermo-Fisher Scientific). Cells were visualized under inverted fluorescence microscope (Olympus IX70), and analysed with Image-Pro Plus software v.7.0 (Media Cybernetics).

3.7. Mycoplasma test

Mycoplasma detection was performed by PCR using primers listed in Table 3.

3.8. Radio-sensitivity test

Sensitivity to IR of AT-iPS clones was examined relating to that of healthy donor (HD)-derived iPSC. Briefly, on Matrigel-coated plates, cells were treated with 8 Gy, and incubated at 37 °C and 5% CO_2 for 1 h. Then, cells were detached with Accutase (Thermo-Fisher Scientific), permeabilized with Reagent A (3 min at RT, SIC) and cold methanol (10 min at 4 °C), centrifuged, and washed twice in PBS-FCS 5%. Cells were incubated with gently shaking for 1 h, at RT, in Reagent B (SIC) with anti-phospho-Histone H2A.X (Ser139) and anti-phospho-ATM (Ser1981) (Merck-Millipore, Table 3). After washing, cells were collected by flow-cytometry, and data analysed with FlowJo software v8.8.6. Based on mean fluorescence intensity assess, signal of treated-iPSCs was normalized subtracting the basal phosphorylation value obtained in not-treated cells.

Declaration of Competing Interest

None.

Acknowledgments

We wish to thank the patient and her family for the collaboration.
We acknowledge the contribution of Fondazione Angelo Nocivelli of Brescia.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:doi:10.1016/j.scr.2019.101596.

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