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Lab Resource: Multiple Cell Lines

Generation of human pluripotent stem cell lines (iPSCs) from mesenchymal stem cells (MSCs) from three elderly patients with osteoarthritis

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A B S T R A C T

Mesenchymal stem cells (MSCs) are a unique population of adult stem cells that can differentiate into many cell types. As such, MSCs represent an interesting source of stem cells for use in the clinical treatment of a variety of disorders involving tissue regeneration. It is therefore crucial to investigate further, whether MSCs from patients with bone or cartilage diseases are able to provide iPSCs lines with efficient differentiation ability into MSC derivatives. For this purpose, we derived 3 stable iPSC lines from the MSCs of 3 elderly patients with osteoarthritis (OA) able to re-differentiate into MSC to make bone, cartilage and adipose tissue.

Resource Table:

Unique stem cell lines identifier	REGUi006-A REGUi007-A REGUi008-A
Alternative names of stem cell lines	IPSC-MSC PEN *(REGUi006-A)* IPSC-MSC DAP *(REGUi007-A)* IPSC-MSC 1514 *(REGUi008-A)*
Institution	CHU Montpellier, Saint Eloi Hospital
Contact information of distributor	dr..Lemaitre, jean-marc.lemaitre@inserm.fr
Type of cell lines	Human induced pluripotent stem cells (hiPSC)
Origin	human
Cell Source	MSC
Clonality	mixed
Method of reprogramming	Sendai virus expressing OCT4, SOX2, C-MYC, KLF4 genes
Multiline rationale	Same disease, non-isogenic cell lines
Gene modification	yes
Type of modification	hereditary
Associated disease	Osteoarthritis
Gene/locus	none
Method of modification	none
Name of transgene or resistance	none
Inducible/constitutive system	none
Date archived/stock date	none
Cell line repository/bank	SAFE-iPS facility, IRMB, CHU MONTPELLIER
Ethical approval	approval by the Local Ethical Committee (registration number: DC–2009–1052)

1. Resource utility

Osteoarthritis is a chronic joint disease that affects approximately 3.6% of the world's population (Cross et al., 2014). Despite several GWAS analyses, the (epi)genomic nature of the pathology is still elusive. To further define early molecular and cellular events involved in the progression of the pathology, it is crucial to find signatures of the pathology on MSC at the origin of the cell derivatives forming bone and cartilage, as potential targets to develop therapeutic or treat the pathology. iPSCs derived from OA MSC re-differentiated into iMSC are a valuable tool to both discriminate between genetic or epigenetic origin of the disease and to identify early events in the appearance of the pathology.

2. Resource details

Three MSC lines were obtained from elderly people of 64 years (MSC PEN and 1514) and 60 years (MSC DAP) with osteoarthritis. IPSC line were derived from those MSCs, using a non integrative method using sendai viruses bearing the 4 reprogramming yamanaka factors (Takahashi et al., 2017), in the presence of irradiated NUFF1 human fibroblasts and using a specific "Pluriton" medium. Then, the iPS-MSCs were mechanically recovered and further grown on matrigel with a

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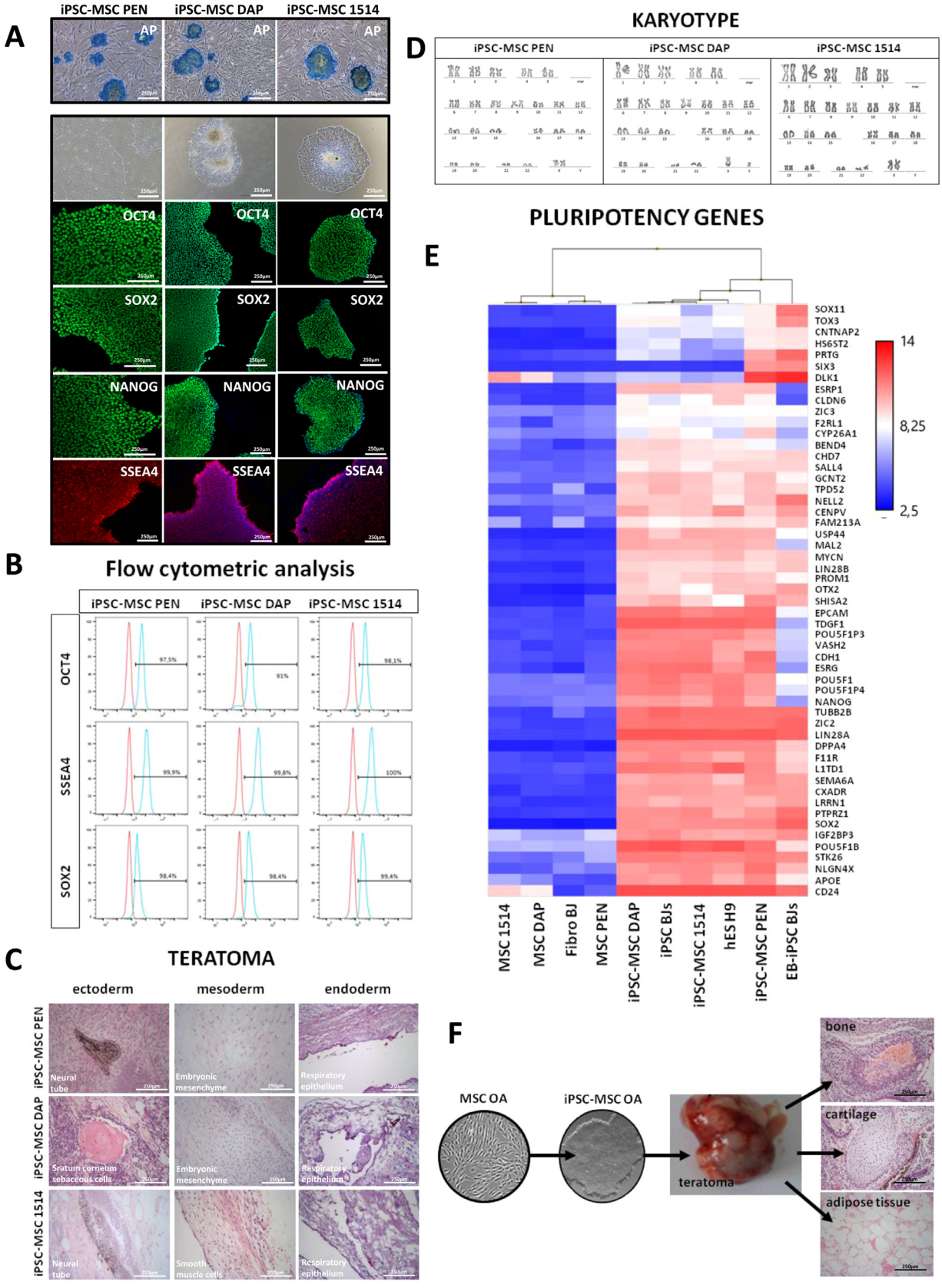
A
iPSC-MSC PEN
iPSC-MSC DAP
iPSC-MSC 1514
AP
OCT4
SOX2
NANOG
SSEA4
B
Flow cytometric analysis
iPSC-MSC PEN
iPSC-MSC DAP
iPSC-MSC 1514
OCT4
SSEA4
SOX2
97,5%
91%
98,1%
99,9%
99,8%
100%
98,4%
98,4%
99,4%
C
TERATOMA
ectoderm
mesoderm
endoderm
iPSC-MSC PEN
iPSC-MSC DAP
iPSC-MSC 1514
Neural tube
Embryonic mesenchyme
Respiratory epithelium
Stratum corneum sebaceous cells
Embryonic mesenchyme
Respiratory epithelium
Neural tube
Smooth muscle cells
Respiratory epithelium
D
KARYOTYPE
iPSC-MSC PEN
iPSC-MSC DAP
iPSC-MSC 1514
E
PLURIPOTENCY GENES
SOX11
TOX3
CNTNAP2
HS6ST2
PRTG
SIX3
DLK1
ESRP1
CLDN6
ZIC3
F2RL1
CYP26A1
BEND4
CHD7
SALL4
GCNT2
TPD52
NELL2
CENPV
FAM213A
USP44
MAL2
MYCN
LIN28B
PROM1
OTX2
SHISA2
EPCAM
TDGF1
POU5F1P3
VASH2
CDH1
ESRG
POU5F1
POU5F1P4
NANOG
TUBB2B
ZIC2
LIN28A
DPPA4
F11R
L1TD1
SEMA6A
CXADR
LRRN1
PTPRZ1
SOX2
IGF2BP3
POU5F1B
STK26
NLGN4X
APOE
CD24
14
8,25
2,5
MSC 1514
MSC DAP
Fibro BJ
MSC PEN
iPSC-MSC DAP
iPSC BJs
iPSC-MSC 1514
hES H9
iPSC-MSC PEN
EB-iPSC BJs
F
MSC OA
iPSC-MSC OA
teratoma
bone
cartilage
adipose tissue

Fig. 1.

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
hiPSC-MSC PEN	iPSC-MSC PEN	Female	64	Caucasian	N/A	Osteoarthritis
hiPSC-MSC DAP	iPSC-MSC DAP	Male	60	Caucasian	N/A	Osteoarthritis
hiPSC-MSC 1514	iPSC-MSC 1514	Female	64	Caucasian	N/A	Osteoarthritis

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	All hiPSC-MSC lines appeared normal	*Fig. 1 panel A*
Phenotype	Qualitative analysis (Immunocytochemistry)	All iPSC-MSC lines are positive for pluripotency markers **Oct4, Nanog, Sox2, SSEA4**, Tra1–81, Tra–1–60 (not shown)	*Fig. 1 panel A*
	Quantitative analysis (Flow Cytometry)	All iPSC-MSC lines are positive for cell surface markers **OCT3/4** (PEN–97,%; DAP–91%; 1514–98%) **SSEA-4** (PEN-99,9% ; DAP-99,8% ; 1514–100%) **SOX2** (PEN-98,4% ; DAP-98,4% ; 1514–99,4%)	*Fig. 1 panel B*
Karyotype	Karyotype (RHG banding)	*46XY, Resolution 400*	*Fig. 1 panel D*
Identity	STR analysis	DNA Profiling 16 STR were site tested, and matched between the original cell lines and the reprogrammed ones	*Supplementary Table 1*
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma testing by luminescence (MycoAlert Mycoplasma Detection Kit, Lonza).	All hiPSC-MSC lines are negative	*Supplementary Table 2*
Differentiation potential	Teratoma formation	Detection of the presence of the three embryo germ layer by histochemistry (validated by a certified Anatomo-histopathologist)	*Fig. 1 panel C*
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative by Elisa analysis	Not shown but available
Genotype additional info (OPTIONAL)	Blood group genotyping	Not performed	N/A
	HLA tissue typing	Not performed	N/A

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-citometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers immunostaining	Oct-4A Rabbit mAb (Clone C30A3) IgG	1:200	Cell signaling technology cat#2840, RRID: AB_2,167,691
Pluripotency markers immunostaining	Sox2 XP® Rabbit mAb (Clone D6D9) IgG	1:200	Cell signaling technology cat# 3579, RRID: AB_2,195,767
Pluripotency markers immunostaining	Nanog XP® Rabbit mAb (Clone D73G4) IgG	1:200	Cell signaling technology cat# 4903, RRID: AB_10,559,205
Pluripotency markers immunostaining	SSEA4 Mouse mAb (Clone MC813) IgG	1:200	Cell signaling technology cat# 4755, RRID: AB_1,264,259
Pluripotency markers immunostaining	TRA–1–60(S)IgM Mouse mAb (Clone TRA–1–60(S)) IgM	1:200	Cell Signaling Technology Cat# 4746, RRID: AB_2,119,059
Pluripotency markers immunostaining	TRA–1–81 Mouse mAb (Clone TRA–1–81) IgM	1:200	Cell signaling technology cat# 4745, RRID: AB_2,119,060
Pluripotency markers immunostaining	Alexa Fluor® 488 conjugate Goat anti-Rabbit IgG	1:400	fisher scientific cat# A-11,034, RRID: AB_2,576,217
Pluripotency markers immunostaining	Secondary Antibody Alexa Fluor® 555 conjugate Goat anti-Rabbit IgG	1:400	Invitrogen-thermofisher scientific cat#A-21,424, RRID: AB_141,780
Pluripotency markers flow cytometry	PE Mouse anti-human Nanog (Clone: N31–355)	1:5	BD Biosciences Cat#560,791, RRID: AB_1,937,305
Pluripotency markers flow cytometry	PerCP-CyTM 5.5 Mouse anti-Oct3/4 (Clone: 40/Oct-3)	1:5	BD Biosciences Cat#560,794, RRID: AB_1,937,313
Pluripotency markers flow cytometry	Alexa FluorR 647 Mouse anti-Sox2 (Clone:245,610)	1:5	BD Biosciences Cat# 560,301, RRID: AB_1,645,308
Pluripotency markers flow cytometry	Alexa FluorR 647 Mouse anti-SSEA-4 (Clone: MC813–70)	1:5	BD Biosciences Cat#560,796, RRID: AB_2,033,991
Pluripotency markers flow cytometry	PE Mouse IgG1, κ Isotype Control (Clone MOPC-21)	1:5	BD Biosciences Cat#554,121, RRID: AB_395,252
Pluripotency markers flow cytometry	PerCP-Cy5.5 Mouse IgG1, κ Isotype Control (Clone: X40)	1:5	BD Biosciences Cat#347,202, RRID: AB_400,265
Pluripotency markers flow cytometry	Alexa Fluor® 647 Mouse IgG2a, κ Isotype Control (Clone: MOPC-173)	1:5	BD Biosciences Cat#558,020, RRID: AB_396,989

specific medium E8 medium. The exhibit normal morphology (Fig. 1A - Characterization of iPSC-MSC colonies, morphology (bright field image), are positive for alkaline phosphatase staining and for specific pluripotency markers, analyzed by immunofluorescence and flow cytometry analysis (Fig. 1B). The identity of iPSC-MSC was confirmed and compared to parental cells by STR analysis. The iPSC-MSC showed a normal and stable diploid karyotype (46, XY) (Fig. 1D and supplementary Fig. 1). Pluripotency was assessed by the ability of iPSC-MSC to differentiate into three germ layers by teratoma formation after injection in immunocompromised mice (Fig. 1C-Teratoma on iPSC-MSC analyzed after hematoxylin eosin saffron (HES) staining of histological sections). Teratoma allowed to observe the ability of these iPSC-MSC to differentiate into bone, cartilage and adipose tissue derivatives (Fig. 1F). In addition, pluripotency was confirmed by re-expression of pluripotency genes comparing fibroblasts/embryoid bodies and different iPSCs (Fig. 1E-Heatmap of transcriptome of iPSC-MSCs, analyzed by supervised clustering of genes involved in pluripotency: the list of genes involved in pluripotency was described in Guenther et al., 2015. Transcriptomic analysis was performed on fibroblasts BJ (Fibro BJ), MSC-PEN, MSC-DAP, MSC-1514, iPSC BJ derived from BJ fibroblasts reprogrammed with sendai viruses, the corresponding embryoid bodies harvested at 15 days of differentiation (EB iPSC BJ), iPSCs obtained from OA MSCs (iPSC-MSC PEN, iPSC-MSC DAP and iPSC-MSC 1514), a human embryonic pluripotent stem cell line (hES H9).

3. Materials and methods

3.1. Reprogramming MSCs into iPSCs

MSCs were isolated from adult bone marrow using standard procedures and cultured in αMEM (Cat#BE12-169F, Lonza), 2 mM GlutaMAX™ (Cat#35,050,038, ThermoFisher Scientific), 10% FCS qualified for MSC culture (Cat#12,662,029, Life Technologies), alpha-FGF 1 ng/ml (Cat#100–18B, Peprotech). The 70% confluent MSCs were transduced by the Sendai virus using the Sendai CytoTune®-iPS 2.0 reprogramming kit (Cat#A34546, Thermo Fisher Scientific) according to the Certificate of Analysis and Miere et al. (2016). On day 7, the cells being reprogrammed were passaged in TrypLE (Cat#12,604,021, Thermo Fisher Scientific), centrifuged and cell pellet was resuspended in conditioned Pluriton medium. The cell suspension was distributed in dishes previously coated with irr-NUFF1. The medium was changed daily to allow iPSC colonies to grow and expand in culture. After 3 weeks, ES-like colonies were isolated manually.

IPSC-MSCs were maintained on extracellular matrix Matrigel (Cat#354,277, Fisher Scientific) in Essential8™ culture media (Cat#A15169-01, Thermo Fisher Scientific), according to the manufacturer's instruction at 37 °C in 5% O2 and 5% CO2.

3.2. Karyotyping

It was performed as previously described (Gatinois et al., 2019), using standard procedures at ChromoStem facility of Montpellier (Fig. 1D).

3.3. Short tandem repeat analysis (STR)

Analysis was carried as previously described (Gatinois et al., 2020), on a list of common STR (Additional data).

3.4. Mycoplasma detection

Mycoplasma is detected with MycoAlert® Detection Kit (Lonza) according to manufacturer's instructions.

Tables 1 and 2

3.5. Flow cytometry analysis

Cells were analyzed on a CANTO II Becton Dickinson and analysis was made with Flow-JO, after treatment of the cells with BD Stemflow Human Pluripotent Stem Cell Transcription Factor Analysis Kit. Results are presented in Fig. 1B and antibodies used are in Table 3.

3.6. Immunofluorescence

Cells grown on coverslips were fixed in 4% paraformaldehyde in PBS and incubated overnight at room temperature, with antibodies specific for pluripotency markers, after 0,1% Saponin permeabilization in the blocking buffer (5% goat serum) according to the standard protocol of StemLightTM Pluripotency Antibody Kit (Cell Signaling, Cat#9656). Antibodies panel are listed in Table 3. Appropriate fluorochrome-conjugated anti primary antibodies with Alexa Fluor® 488 and Alexa Fluor® 555 dyes were applied. DNA was counterstained with DAPI (Cat#6244, ImmunoChemistry) 15 min and coverslips mounted in Vectashield (Vector, Cat#H-1400). Image acquisition was performed with an Axio Imager Z1(ZEISS) Apotome, X10 objective (Fig. 1A).

3.7. Transcriptomic analysis

Total RNA isolation was performed using the RNeasy Mini Kit (Cat#52,304, Qiagen), according to manufacturer's instructions. RNA was hybridized on a Affymetrix GeneChip Human Genome U133 Plus 2.0 array (Fig. 1E).

3.8. Teratoma formation

The differentiation potency was performed by *in vivo* teratoma formation. Cell clusters corresponding to approximately 3×10^6 iPSCs were injected subcutaneously into anesthetised NOD-SCID-gamma mice (NOD.CgPrkdcscidIl2rgtm1Wjl/SzJ). Mices were transplanted in dorsolateral area on both sides at 6–8 weeks old. After 8–10 weeks of latency, teratomas were fixed, embedded in paraffin blocks, stained by hematoxylin eosin saffron (HES) and analyzed by a pathologist (RHEM, Montpellier, France) for the presence of structures from the 3 embryonic germ layers (Fig. 1C).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2020.101721.

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