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Establishment of a collection of human pluripotent stem cell lines (iPSC) from mesenchymal stem cells (MSC) from three healthy elderly donors

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ABSTRACT

The study of molecular mechanism driving osteoarticular diseases like osteoarthritis or osteoporosis is impaired by the low accessibility to mesenchymal stem cells (MSC) from healthy donors (HD) for differential multi-omics analysis. Advances in cell reprogramming have, however, provided both a new source of human cells for laboratory research and a strategy to erase epigenetic marks involved in cell identity and the development of diseases. To unravel the pathological signatures on the MSC at the origin of cellular drifts during the formation of bone and cartilage, we previously developed iPSC from MSC of osteoarthritis donors. Here we present the derivation of three iPSCs from healthy age matched donors to model the disease and further identify (epi)genomic signatures of the pathology.

1. Ressource table

Unique stem cell lines identifier	REGUI012-A REGUI013-A REGUI014-A
Alternative names of stem cell lines	IPSC-MSC56 (REGUI012-A) IPSC-MSC58 (REGUI013-A) IPSC-MSC119 (REGUI014-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	control
Gene modification	yes
Type of modification	hereditary
Associated disease	none
Gene/locus	none
Method of modification	none
Name of transgene or resistance	none
Inducible/constitutive system	none

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(continued)

Date archived/stock date	none
Cell line repository/bank	SAFE-iPSC facility, IRMB, CHU MONTPELLIER
Ethical approval	Approval by the Local Ethical Committee (registration number: DC-2009-1052)

2. Ressources utility

Osteoarthritis (OA) and osteoporosis (OP) are skeletal diseases that affects more than 200 Millions people worldwide for each (Al Maini et al., 2020). To better define the cellular and molecular events involved in the progression of the pathologies, we have to further identify pertinent (epi)genomic signatures of the pathology on MSCs, as potential targets to develop innovative therapeutic strategy. To further model OA and OP and to discriminate the genetic or epigenetic etiology of the diseases and to identify the early events involved in the appearance of OA or OP, a comparison of gene expression and epigenetic marks of MSC from HD and OA and OP donors as well as MSC derived from iPSC (HD and OA, OP) has to be investigated (See Table 1 and 2 and Pichard et al., 2020).

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Table 1

Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
hiPSC-MSC 56	iPSC-MSC 56	Male	76	Caucasian	N/A	none
hiPSC-MSC 58	iPSC-MSC 58	Male	78	Caucasian	N/A	none
hiPSC-MSC 119	iPSC-MSC 119	Male	76	Caucasian	N/A	none

3. Resource details

Three MSC lines were obtained from healthy donors of 76-year-old (MSC56), 76-year-old (MSC119) and 78-year-old (MSC58). We thank Dr Sandra Noack and Christian Krettek from Medizinische Hochschule Hannover (MHH) for their generous gift. The iPSC lines were derived from those MSCs, using a non-integrative method using sendai viruses bearing the 4 reprogramming Yamanaka factors (Guenther et al., 2010), in the presence of irradiated human fibroblasts NUFF1 and using a specific “Pluriton” medium. Then, the iPSC-MSCs were mechanically recovered and cultivated on matrigel with a specific E8 medium. The resulting iPSC-MSCs showed a normal morphology (Fig. 1A - Characterization of the iPSC-MSC colonies - bright field image), a positive staining with alkaline phosphatase and for specific pluripotency markers analyzed by immunofluorescence and by flow cytometry (Table 3 and Fig. 1B). The identity of the iPSC-MSCs was confirmed by comparison with the parental cells by STR analysis (Table 1 Supplementary) and the genetic stability was validated by a normal. CGH-Array (Table 3 Supplementary). The pluripotency was evaluated by the ability of iPSC to differentiate into the three germ layers by teratoma formation after injection in immunocompromised mice (Fig. 1C-Teratoma on iPSC-MSC analyzed after haematoxylin eosin saffron (HES) staining of the histological sections). Teratomas offer the opportunity to validate the ability of iPSCs to differentiate, into bone and cartilage derivatives. In addition, pluripotency was confirmed by the re-expression of pluripotency genes (Fig. 1D-Heatmap of the iPSC transcriptome), analyzed by supervised clustering of genes involved in pluripotency. The list of genes involved in pluripotency was described previously (Guenther et al., 2010). The transcriptomic analysis was carried out on fibroblasts BJ (Fibro BJ), iPSC-BJ derived from BJ fibroblasts reprogrammed with sendai viruses,

a human embryonic pluripotent stem cell line (hESC-H9) and MSC-HD (MSC56, MSC58 and MSC119) and the iPSC obtained from MSC-HD, as the collection of healthy iPSCs (iPSC-MSC56, iPSC-MSC58 and iPSC-MSC119).

4. Materials and methods

4.1. Reprogramming MSCs into iPSCs

MSCs were isolated from adult bone marrow using standard procedures and cultured in α MEM (Cat#BE12-169F Lonza) 2 mM Gluta-MAX™ (Cat#35050038 ThermoFisher Scientific) 10% FCS qualified for MSC culture (Cat#12662029 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 are passaged in TrypLE (Cat#12604021 Thermo Fisher Scientific) centrifuged and cell pellet is resuspended in conditioned Pluriton medium. The cell suspension is distributed in dishes previously coated with irr-NUFF1. The medium is changed daily to allow iPSC colonies to grow and expand in culture. After 3 weeks ES-like colonies were isolated manually HiPsc were maintained on extracellular matrix Matrigel (Cat#354277, 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.

4.2. Short tandem repeat analysis (STR) (Supplementary Table 1)

Analysis was carried out with GeneMarker V2.6.7 (SoftGenetics) on a list of common STR.

4.3. Mycoplasma detection (Supplementary Table 2)

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

4.4. Genomic DNA extraction and ACPA analysis (Supplementary Table 3)

DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen), following the manufacturer's instructions. Genome variation profiling by chromosomal microarray was conducted at ChromoStem facility (Montpellier, France, <http://www.chumontpellier.fr/fr/cherche>

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, Tra-1-81, 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 (56–97%; 58–100%; 119–98.2%) SSEA-4 (56–100%; 58–100%; 119–100%) SOX2 (56–97.4%; 58–98.6%; 119–92%)	Fig. 1 panel B
Genotype	CGH array	All hiPSC-MSC lines appeared normal	Supplementary Table 3
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
Microbiology and virology	Southern Blot OR WGS	N/A	N/A
	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 Anatomico-histopathologist)	Fig. 1 panel C
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	not shown by trauma surgery clinic, Hannover
Genotype additional info (OPTIONAL)	Blood group genotyping	Not Performed	N/A
	HLA tissue typing	Not Performed	N/A

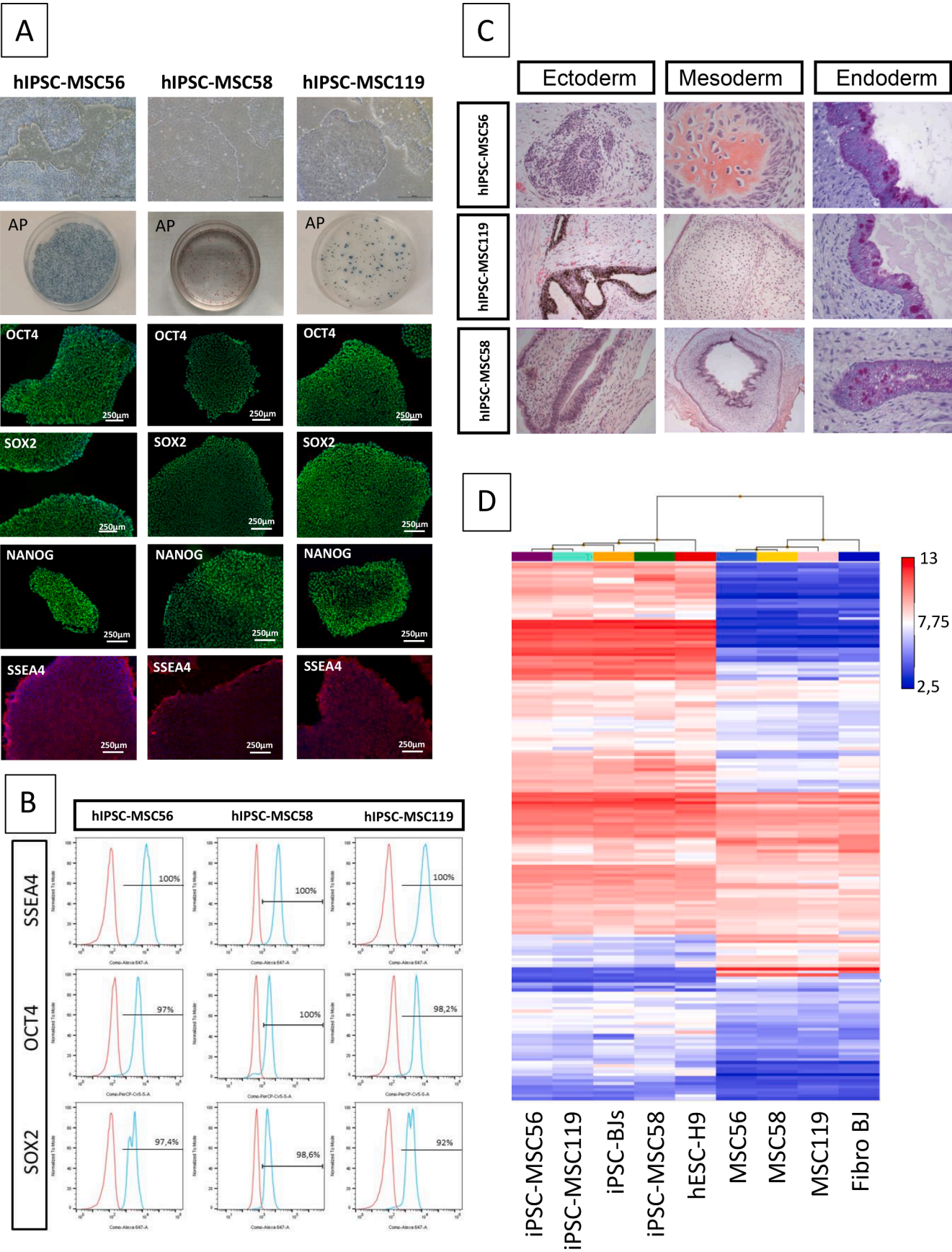


Fig. 1.

Table 3
Reagents details.

Antibody	Dilution	Company Cat # and RRID
Oct-4A Rabbit mAb (clone C30A3) IgG	1:200	Cell Signaling Technology Cat# 2840, RRID:AB_2167691
Sox2 XP Rabbit mAb (clone D6D9) IgG	1:200	Cell Signaling Technology Cat# 3579, RRID:AB_2195767
Nanog XP Rabbit mAb (clone D73G4) IgG	1:200	Cell Signaling Technology Cat# 4903, RRID:AB_10559205
SSEA4 Mouse mAb (clone MC813) IgG	1:200	Cell Signaling Technology Cat# 4755, RRID:AB_1264259
TRA-1-60(S) IgM Mouse mAb (clone TRA-1-60(S)) IgM	1:200	Cell Signaling Technology Cat# 4746, RRID:AB_2119059
TRA-1-81 Mouse mAb (clone TRA-1-81) IgM	1:200	Cell Signaling Technology Cat# 4745, RRID:AB_2119060
Secondary Antibody Alexa Fluor 488 conjugate Goat anti-Rabbit IgG	1:400	Fisher Scientific Cat#A-11034, RRID:AB_2576217
Secondary Antibody Alexa Fluor 555 conjugate Goat anti-Rabbit IgG	1:400	Invitrogen Thermofisher Scientific Cat#A-21424, RRID:AB-141780
PE Mouse anti-human Nanog (clone N31-355)	1:5	BD Biosciences Cat#560791, RRID:AB_1937305
PerCP-CyTM 5.5 Mouse anti-Oct3/4 (clone 40/Oct3)	1:5	BD Biosciences Cat#560794, RRID:AB_1937313
Alexa FluorR 647 Mouse anti-Sox2 (clone 245610)	1:5	BD Biosciences Cat#560301, RRID:AB_1645308
Alexa FluorR 647 Mouse anti-SSEA-4 (clone MC813-70)	1:5	BD Biosciences Cat#560796, RRID:AB_2033991
PE Mouse IgG1, k isotope Control (clone MOPC-21)	1:5	BD Biosciences Cat#554121, RRID:AB_395252
PerCP-Cy5.5 Mouse IgG1, k isotope Control (clone X40)	1:5	BD Biosciences Cat#347202, RRID:AB_400265
Alexa Fluor 647 Mouse IgG2a, k isotope Control (clone MOPC-173)	1:5	BD Biosciences Cat#558020, RRID:AB_396989

urs/plateformes/les-plateformes) with a SurePrint G3 Human CGH Microarrays 8x60k (Agilent ®), and no additional CNV was detected in comparison to parental cells.

4.5. Flow cytometry analysis

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

4.6. 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 StemLight™ 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).

4.7. Transcriptomic analysis

Total RNA isolation was performed using the RNeasy Mini Kit (Cat#52304, Qiagen), according to manufacturer's instructions. RNA was hybridized on a Affymetrix GeneChip Human Genome U133 Plus 2.0 array (Fig. 1D).

4.8. 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 anesthetized NOD-SCID-gamma mice (NOD.CgPrkdcscidIl2rgtm1Wjl/SzJ). Mice were transplanted in dorso-lateral 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).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scr.2021.102297>.

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