Journal Pre-proofs

Generation of normal induced pluripotent stem cell line KUMCi002-A from bone marrow CD34+ cells of patient with multiple myeloma disease having 13q deletion and IGH translocation

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patient with multiple myeloma disease having 13q deletion and IGH translocation

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Abstract: Multiple myeloma (MM) is a hematological cancer characterized by an uncontrolled proliferation of antibody-secreting plasma cells within the bone marrow. Currently, cell therapy such as chimeric antigen receptor T-cell (CAR-T) based on induced pluripotent stem cells (iPSCs) has received attention for treating MM. However, the generation of iPSCs from MM patients appears to be very rarely reported. Here we generated an iPSC line from CD34+ bone marrow cells of a patient with MM using human placenta-derived cell conditioned medium (hPCCM), offering a relatively high efficiency in humanized conditions. This iPSC line might be a useful model for research on MM.

Resource Table:

Unique stem cell line identifier	KUMCi002-A
Alternative name(s) of stem cell line	MMCD34+_iPSCs
Institution	KOREA UNIVERSTY COLLEGE OF MEDICINE
Contact information of distributor	SeungJin Lee, jin5275@korea.ac.kr
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 49 Sex: Female Ethnicity if known: Korean
Cell Source	MM patient bone marrow CD34+ cells
Clonality	Clonal
Method of reprogramming	Sendai virus harboring Oct4, Sox2, Klf4, and cMyc
Genetic Modification	YES
Type of Modification	Gene Correction (deletion and translocation)
Associated disease	Multiple myeloma
Gene/locus	N/A
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	August 2020
Cell line repository/bank	Registered in the Human Pluripotent Stem Cell Registry (https://hpscreg.eu)
Ethical approval	The study has been approved by the Institutional Review Board of Anam Hospital of the Korea University Medical Center. (#2020AN0067)

Resource utility

This resource will be valuable to enhance the reprogramming of human CD34+ cells to human induced

drugs.

Resource Details

Reports showing the development of hiPSC line from MM patients appear to be very rare. Recently, we developed human placenta-derived cell conditioned medium (hPCCM) accelerating the reprogramming of human somatic cells to hiPSCs under humanized conditions (Lee et al., 2020a) and reported the generation of a hiPSC line from a patient with acute lymphoblastic leukemia (Lee et al., 2020b). Therefore, herein we established a hiPSC line from a patient with MM using the same method. Human primary bone marrow CD34+ cells were isolated from patients with MM harboring 13q: IGH/CCND1 deletion and transduced with Oct4, Sox2, Klf4, and cMyc Sendai viruses for reprogramming into iPSCs. After transduction, the cells were transferred onto 0.1% gelatin coated dish and cultured in hPCCM as previously described (Fig 1A) (Lee, 2020). Generated iPSC colonies were confirmed by using ALP and TRA-1-60 staining among the colonies showing hiPSCs morphology, which was picked manually 7 days after the transduction. Thereafter, the colonies of hiPSCs from bone marrow CD34+ cells of a patient with MM (MMCD34+_iPSCs) were expanded in feeder-free culture condition (Fig 1A). To validate the identity, short tandem repeat (STR) analysis was performed (Supplementary Table 1), and the expression of pluripotency markers OCT4, Nanog, and SSEA4 was confirmed by immunofluorescence (Fig 1B). MMCD34+_iPSCs showed the morphology of undifferentiated iPSCs and expressed pluripotency genes, which was confirmed by comparing with normal iPSCs as a positive control and the patient's MM_CD34+ cells as a negative control (Fig 1C). Furthermore, the embryoid bodies of MMCD34_iPSCs were able to differentiate into the three germ layers in vitro, as confirmed by the expression of AFP and FOXA2 for endoderm, DESMIN for mesoderm, and NESTIN and TUJ1 for ectoderm (Fig 1D). After 7 passages, the karyotype of the MMCD34+_iPSCs was inspected by G-banding and the absence of Sendai virus was confirmed by RT-qPCR using specific primers. Cytogenetic analysis of MMCD34+_iPSCs showed a normal karyotype after the elimination of Sendai virus, despite the presentation of the 13q deletion before transduction (Fig 1E and F and Supplementary Figure 1). These findings indicate that it is possible to generate karyotypically normal iPSCs from the CD34+ bone marrow cells of MM patients. In addition, mycoplasma detection revealed that this iPSC line has not been infected by mycoplasma (Supplementary Figure 2).

Materials and Methods

Ethics

This study and the experimental procedures conducted were approved by the Institutional Review Board of Anam Hospital of the Korea University Medical Center, and informed consent was obtained from donors in accordance with the Declaration of Helsinki. (IRB#2020AN0067)

Cell culture

CD34+ cells from the bone marrow of MM patient were isolated using magnetic bead separation system (Miltenyi Biotec) and cultured in Stemline II Hematopoietic Stem Cell Expansion Medium (Sigma-Aldrich) containing 100 ng/mL stem cell factor (SCF), thrombopoietin (TPO), and G-CSF (R&D Systems, MN, USA). The medium was changed every 2 days and the culture was continued until 14 days. Normal bone marrow derived human iPSCs (IISH1i-BM1) were purchased from WiCell Research Institute (Madison, WI, USA) and handled in accordance with the manufacturer's instructions; the medium was changed daily and the cells were passaged every 4–5 days. All the cells were cultured at 37 °C in an atmosphere of 5% CO_2.

Human placenta-derived cell conditioned medium

The placental cells were isolated from placental chorionic plates and cultured in DMEM containing 10% fetal bovine serum (Gibco) at 37 °C in an atmosphere of 5% CO_2. At 80% confluency, the cells were harvested in conditioned medium after 24 h of incubation in DMEM-F12 supplemented with 20% Knockout™ Serum

−80 °C.

Generation of iPSCs

MM_CD34+ cells were generated using the CytoTune-iPS 2.0 Sendai Reprogramming Kit (Life Technologies) according to the manufacturer's instructions. Briefly, CD34+ cells (1×10^5) were transduced with Sendai viruses at MOI = 5 in Stemline II Hematopoietic Stem Cell Expansion Medium supplemented with 100 ng/mL SCF, 100 ng/mL TPO, and 50 ng/mL G-CSF. A day after transduction, the cells were transferred onto a 0.1% gelatin-coated 35-mm dish in hPCCM. On the subsequent day, half of the medium was replaced with fresh hPCCM and the entire medium was changed every day until day 7. After picking iPSC colonies, iPSCs were cultured and expanded on Matrigel-coated dishes in mTeSR™1 (STEMCELL Technologies) at 37 °C in an atmosphere of 5% CO_2. To confirm pluripotency, cells were live stained with a TRA1–60 antibody (Stemgent).

Detection of alkaline phosphatase activity

Alkaline phosphatase (ALP) activity was detected using the ES Cell Characterization Kit (Millipore) according to manufacturer's instructions.

Immunofluorescence staining

Cells were fixed with 4% paraformaldehyde for 10 min, permeabilized with 0.1% Triton X-100, and blocked for 1 h with 3% normal horse serum (Gibco; Thermo Fisher Scientific). Cells were then incubated with primary antibodies for Oct3/4, SSEA4, and SOX2 (Table 2). Images were acquired using a fluorescence microscope (Olympus). Scale bars represent 200 μm.

Reverse transcription qPCR

qPCR (Bio-Rad iCycler iQ system) was performed using iQ SYBR Green qPCR Master Mix (Bio-Rad). Primers used for qPCR are listed in Table 2. The cycle threshold values of the genes of interest were normalized to that of GAPDH.

Karyotyping

The MM_CD34+_iPSCs at passage 7 were cultured in a feeder-free system for 3 days and dissociated with Accutase (GIBCO). Thereafter, the cells were incubated in 0.075 M KCl for 20 min at 37 °C. After fixation with a 3:1 solution of methanol/acetic acid, the karyotype was determined; at least 20 metaphase spreads were examined by standard G-banding analysis at a 300-band resolution.

Fluorescence In Situ Hybridization

FISH analysis of MM CD34+ iPSCs from patients was performed at the Department of Diagnostic Examination, Anam Hospital of the Korea University Medical Center.

Short tandem repeat genotyping

STR analysis of the generated MM CD34+ iPSCs and parental CD34+ cells was performed using a PowerPlex 16 System (Promega) to detect 16 loci at the Dawoojin Gene Research Institute (Seoul, Korea).

Differentiation

For verifying differentiation into the three germ layers, undifferentiated colonies of hiPSCs were cultured in low-attachment surface plates with DMEM-F12 medium containing 20% knockout serum replacement for 7 days, seeded onto gelatin-coated dishes, and cultured for 7 days.

Mycoplasma test

Mycoplasma test was performed using the e-Myco™ Mycoplasma PCR Detection Kit (iNtRON) according to manufacturer's instructions to confirm the absence of mycoplasma.

Acknowledgements

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References

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Additional files:

Figure 1
Figure 1 is uploaded as TIF file.

Table 1 and Table 2
Table1 and Table2 are uploaded as word files.

STR analysis
STR analysis is uploaded as TIF file.

Supplementary files
Supplementary material (mycotest) is uploaded as TIF file.

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

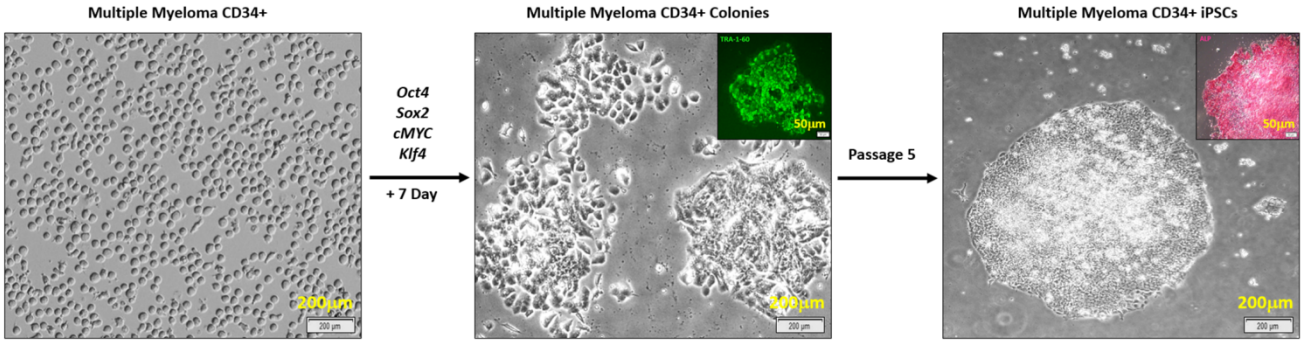
Table 1: Characterization and validation

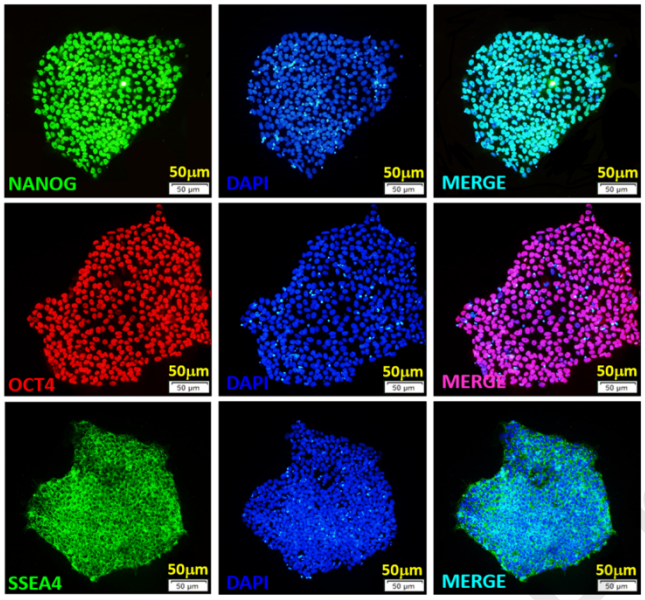
Classification	Test	Result	Data
Morphology	Photography	Normal	Figure 1 panel A
Phenotype	Immunocytochemistry	Positive staining for ALP, OCT4, SOX2, SSEA4, TRA-1-60	Figure 1 panel A and B
	RT-qPCR	Expression of pluripotency markers OCT4, REX1, and SOX2	Figure 1 panel C
Genotype	Karyotype (G-banding) and resolution	46XX, 300 band resolution	Figure 1 panel E
Identity	Microsatellite PCR (mPCR) OR	DNA Profiling not performed	N/A
	STR analysis	16 loci analyzed, all matched	Supplementary Table 1
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	FISH	13q: IGH/CCND1: Negative	Supplementary Figure 1
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR: Negative	Supplementary Figure 2
Differentiation potential	Embryoid body formation (immunofluorescence)	Expression of endodermal (AFP, FOXA2), mesodermal (DESMIN) and ectodermal (NESTIN, TUJ1) genes in embryoid bodies	Figure 1 panel D
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

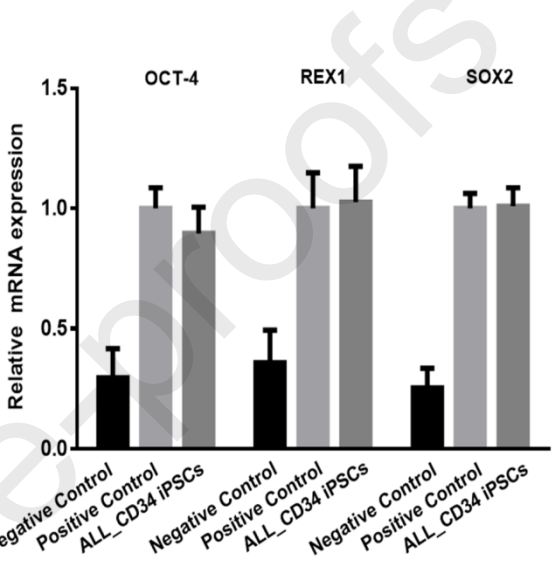
Table 2: Reagent details

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Pluripotency Markers	StainAlive TRA-1-60 (DyLight 488)	1:500	Stemgent Cat# 09-0068, RRID:AB_2233143
Pluripotency Markers	Rabbit anti-OCT4	1:500	Cell Signaling Technology Cat# 2750, RRID:AB_823583
Pluripotency Markers	Rabbit anti-NANOG	1:500	Santa Cruz Biotechnology Cat# sc-293121, RRID:AB_2665475

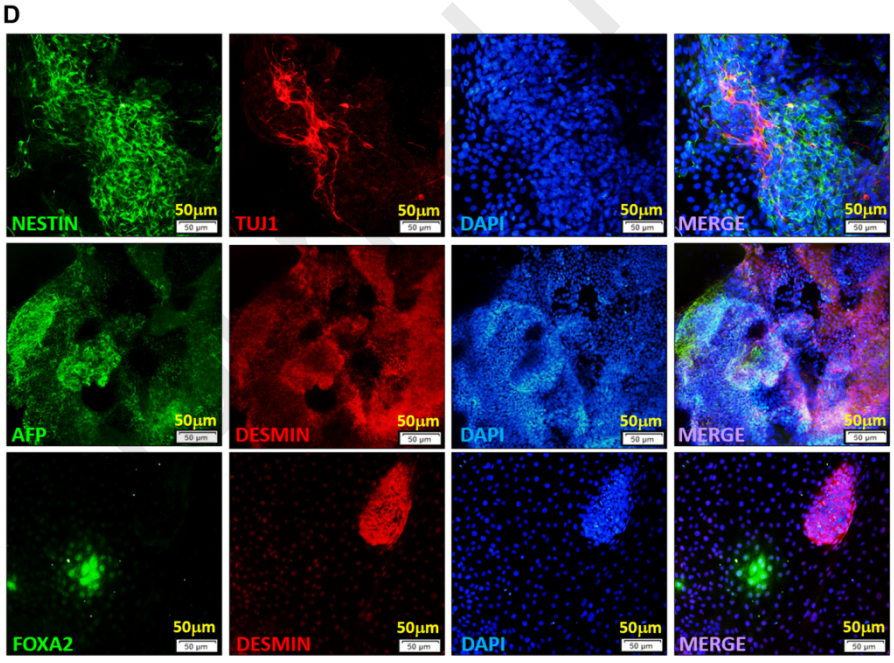
			RRID:AB_177629
Differentiation Markers	Mouse anti-AFP	1:500	Santa Cruz Biotechnology Cat# sc-166335, RRID:AB_2224077
Differentiation Markers	Mouse anti-FOXA2	1:500	Novus Cat# H00003170-M12, RRID:AB_669213
Differentiation Markers	Rabbit anti-DESMIN	1:500	Santa Cruz Biotechnology Cat# sc-14026, RRID:AB_2092608
Differentiation Markers	Mouse anti-NESTIN	1:500	Abcam Cat# ab22035, RRID:AB_446723
Differentiation Markers	Rabbit anti-TUJ1	1:500	Covance Cat# MRB-435P-100, RRID:AB_663339
Secondary antibodies	Goat anti-Rabbit IgG, Alexa Fluor 594	1:1000	Thermo Fisher Scientific Cat# A-11012, RRID:AB_2534079
Secondary antibodies	Goat anti-Rabbit IgG, Alexa Fluor 488	1:1000	Thermo Fisher Scientific Cat# A-11034, RRID:AB_2576217
Secondary antibodies	Goat anti-Mouse IgG, Alexa Fluor 594	1:1000	Thermo Fisher Scientific Cat# A-11005, RRID:AB_2534073
Secondary antibodies	Goat anti-Mouse IgG, Alexa Fluor 488	1:1000	Thermo Fisher Scientific Cat# A-11001, RRID:AB_2534069
Primers			
	Target	**Forward/Reverse primer (5'-3')**	
Pluripotency Markers (qPCR)	OCT4	TCTCGCCCCCTCCAGGT/ CTGCTTCGCCCTCAGGC	
Pluripotency Markers (qPCR)	SOX2	GCGGAAAACCAAGACGCTCA/ GTTCATGTGCGCGTAGCTGT	
Pluripotency Markers (qPCR)	REX1	CAGATCCTAAACAGCTCGCAGAAT/ GCGTACGCAAATTAAAGTCCAGA	
House-Keeping Genes (qPCR)	GAPDH	GAGTCCACTGGCGTCTTCAC/ TTCACACCCATGACGAACAT	
Reprogramming factor silencing (PCR)	SeV	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC	

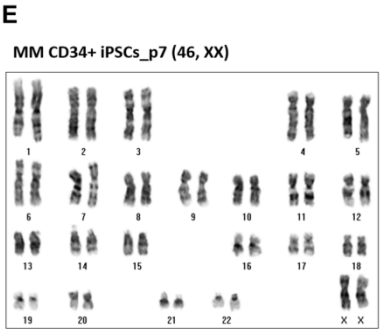


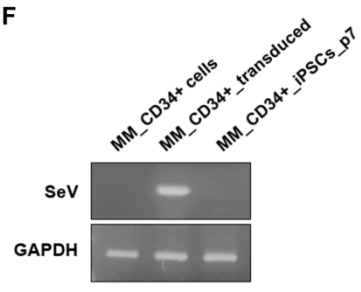




All images at 50X magnification







All images at 50X magnification