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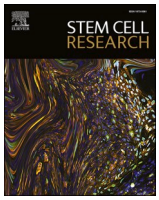

Lab Resource: Single Cell Line

Generation of a human induced pluripotent stem cell line (YUCMi020-A) from peripheral blood mononuclear cells derived from a female with the Jr (a−) blood type

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A B S T R A C T

The Jra antigen, the only antigen within the JR blood group system, is a high-prevalence red blood cell (RBC) antigen found in over 99 % of the global population. An induced pluripotent stem cell line (YUCMi020-A) was generated from peripheral blood drawn from a Jr(a−) phenotype individual, who was homozygous for a null mutation of ABCG2*01N.01 (rs72552713, c.376C>T; p.Gln126*). The generated line exhibited pluripotent characteristics and no chromosomal aberrations. This cell line will serve as a cell source, enabling us to produce RBCs with the Jr(a−) phenotype in vitro, which can be used for transfusing individuals with anti-Jra antibodies.

1. Resource Table

Unique stem cell line identifier	YUCMi020-A
Alternative name(s) of stem cell line	N/A
Institution	Yonsei University College of Medicine
Contact information of distributor	Sinyoung Kim, sykim@yuhs.ac
Type of cell line	Human induced pluripotent stem cell (hiPSC)
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 63 Sex: Female Ethnicity if known: East Asian
Cell Source	Peripheral blood mononuclear cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Episomal (OCT3/4, SOX2, KLF4, L-MYC)
Genetic Modification	No
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	PCR
Associated disease	N/A
Gene/locus	ABCG2*01N.01 (rs72552713, c.376C>T; p.Gln126*)
Date archived/stock date	2022
Cell line repository/bank	https://hpscreg.eu/cell-line/YUCMi020-A
Ethical approval	The study was approved by the Institutional Review Board of Severance Hospital, Yonsei University (IRB No. 4-2018-0890).

2. Resource utility

Due to the high prevalence of the Jra antigen, locating Jr(a−) blood in the general inventory is exceedingly challenging. Consequently, our cell line, derived from an individual with the Jr(a−) phenotype, could serve as a valuable cell source for producing the rare Jr(a−) blood needed for transfusion.

3. Resource details

Blood transfusion is an essential part of modern medicine and plays a crucial role in many medical fields. To maximize the effectiveness of transfusion and ensure patient safety, it is important to provide compatible blood. However, significant challenges may arise in finding compatible blood for individuals who are highly immunized due to frequent transfusions or those with a rare blood type, such as Jr(−).

Jra, the only RBC antigen in the JR blood group system (ISBT 032), is a high-prevalence RBC antigen. The expression of Jra antigen is regulated by the ATP-binding cassette G2 (ABCG2) gene on chromosome 4q22.1 (Toshimitsu et al., 2019). The incidence of the Jr(a−) phenotype varies with race, being relatively prevalent in East Asia, especially in Japan (Kim et al., 2020). The clinical significance of the anti-Jra antibody remains unclear, but several cases reporting mild to severe hemolytic transfusion reactions caused by the anti-Jra have been described

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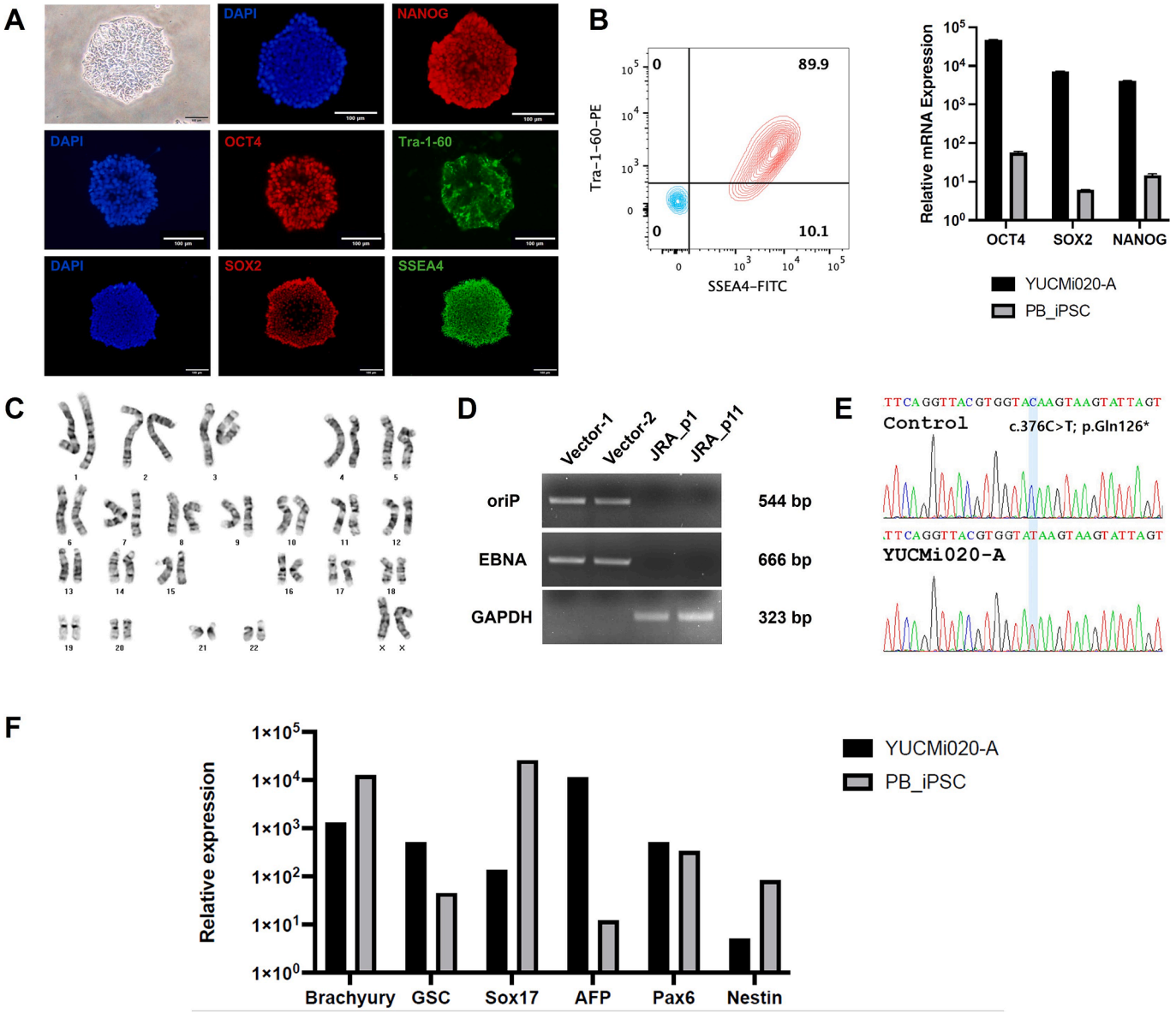

Fig. 1. Characteristics of the hiPSC line. Abbreviation: MNC, mononuclear cells; DC, differentiated cells.

(Kim et al., 2020). When a patient has the anti-Jra antibody and requires RBC transfusion, the optimal approach is to provide Jra antigen-negative RBCs, which are not typically available at the time of patient need. In light of this, developing rare blood type products in vitro would seem like an ideal solution. To achieve this, it is necessary to establish reprogrammed cells and build a registry of hiPSC clones that can be used as a cell source.

In this report, we isolated PBMCs from a 63-year-old female, who was confirmed to have Jr(a−) blood type (Fig. 1D) along with the anti-Jra antibody, and established the YUCMi020-A cell line using episomal vectors. The generated hiPSCs showed a typical iPSCs-like morphology (Fig. 1A) and demonstrated a normal karyotype (Fig. 1C). Immunofluorescence staining, flow cytometry, and qRT-PCR results of the pluripotency markers, Tra-1–60, SSEA4, OCT4, SOX2, and NANOG, confirmed the pluripotent nature of the established hiPSCs (Fig. 1A and 1B). We further verified that our hiPSCs no longer express episomal markers used for reprogramming (Fig. 1D). Directed differentiation of the hiPSCs, followed by the qRT-PCR, revealed the expression of three germ layer markers: PAX6 and NESTIN for ectoderm, BRACHYURY and GSC for mesoderm, and SOX17 and AFP for endoderm (Fig. 1F). The hiPSC line was confirmed to be mycoplasma-free. Short tandem repeat analysis (STR) of the hiPSC line showed a 100 % match with the parental PBMC source in 16 allele loci. Characterizations of YUCMi020-A cells are summarized in Table 1.

4. Materials and methods

4.1. Establishment and maintenance of hiPSCs

hiPSCs were established as previously described (Cho et al., 2023). Briefly, viable PBMCs isolated from a Jr(a−) individual were expanded on erythroid expansion medium. The prepared cells were transfected with the Nepa21 system (Nepa Gene Co., LTD.) using Epi5 Episomal Reprogramming Vectors, Epi p53, and EBNA vectors (Life Technologies), according to the manufacturer's protocols. The transfected cells were cultured on Matrigel-coated plates (14.5 μL Matrigel Matrix (Stem Cell Technologies) and 985.5 μL Dulbecco's Modified Eagle Medium (DMEM)/F12 (1X) (Gibco)) with erythroid expansion medium. hiPSC-like colonies were manually selected and maintained on plates with mTESR Plus Basal media (Stem Cell Technologies) in a 5 % CO_2

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1A
Phenotype	Qualitative analysis by Immunocytochemistry	Positive for NANOG, OCT4, SOX2, Tra-1–60, and SSEA4	Fig. 1A
	Quantitative analysis by flow cytometry and qRT-PCR	Flow cytometry: Positive for Tra-1–60 (89.9 %) and SSEA4 (100 %) qRT-PCR: mRNA expression for OCT4, SOX2 and NANOG	Fig. 1B
Genotype	Karyotype (G-banding) and resolution	46XX, Resolution 550	Fig. 1C
Identity	Microsatellite PCR (mPCR) OR STR analysis	N/A 16 loci analyzed, all matching	N/A Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	Homozygous for ABCG2*01N.01 (rs72552713, c.376C>T; p.Gln126*)	Fig. 1E
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Negative	Supplementary data 1
Differentiation potential	Directed differentiation	Differentiation to all three germ layers confirmed by qRT-PCR	Fig. 1F
List of recommended germ layer markers	Expression of these markers has to be demonstrated at mRNA (RT PCR) or protein (IF) levels, at least 2 markers need to be shown per germ layer	Ectoderm: PAX6, NESTIN Mesoderm: BRACHYURY, GSC Endoderm: SOX17, AFP	Fig. 1F
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping HLA tissue typing	DNA analysis N/A	Fig. 1E N/A

incubator at 37°, with daily medium changes. The cells were enzymatically passaged using ReLeSR (Stem Cell Technologies) every 4–5 days.

4.2. Immunofluorescence assay

The reprogrammed cells, fixed in 4 % paraformaldehyde at room temperature (RT) for 20 min and treated with 0.05 % Tween-20 wash, 0.1 % Triton-100 permeabilization at RT for 15 min, and 4 % Donkey Serum block at 4 °C overnight (all from Sigma-Aldrich), were incubated at 4 °C overnight with primary antibodies (Table 2). Subsequent steps involved washing, incubation at 4 °C overnight with secondary antibodies (Table 2), and staining with UltraCruz Aqueous Mounting Medium with DAPI (Santa Cruz Biotechnology). The cells at passage 11 were visualized using a CKX53 fluorescence microscope (Olympus).

4.3. Fluorescence-activated cell sorting (FACS)

hiPSCs at passage 13 were dissociated using Gentle Cell Dissociation Reagent (Gibco) and stained with conjugated antibodies (Table 2). Unbound antibodies were washed, and the labelled cells were fixed with 4 % formaldehyde. The BD Verse flow cytometer (BD Biosciences) was used for analysis.

4.4. Quantitative RT-PCR

Total RNA was extracted using the RNeasy Plus Mini Kit (Qiagen). Complementary DNA was synthesized using the iScript cDNA Synthesis Kit (Bio-RAD). qRT-PCR was performed using TaqMan Gene Expression Master Mix (Applied Biosystems) following the manufacturer's protocols and analysed using the Step One Plus (Applied Biosystems). The previously established iPSCs derived from a normal individual (PB_iPSC) was used as a control (Cho et al., 2023). The TaqMan probes are listed in Table 2.

4.5. Karyotyping

hiPSCs at passage 11 underwent G-banding karyotyping by GenDX, analysing 20 metaphase spreads using GTG-banding.

4.6. Mycoplasma detection

Mycoplasma detection was performed using the MycoAlert™ PLUS Mycoplasma Detection Kit (Lonza), following the manufacturer's instructions. hiPSCs at passage 13 were tested for analysis.

4.7. Tri-lineage differentiation

hiPSCs were differentiated into three germ-layers using the STEMdiff™ Trilineage Differentiation Kit (Stem Cell Technologies), according to the manufacturer's protocols. The differentiated hiPSCs were analysed by qRT-PCR on Day 5 for mesoderm and endoderm markers and on Day 7 for ectoderm markers.

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency markers	Rabbit anti-OCT4	1:100	Abcam Cat#ab19857	RRID: AB_445175
	Mouse anti-Tra-1–60	1:100	Abcam Cat#ab16288	RRID: AB_778563
	Rabbit anti-NANOG	1:50	Abcam Cat#ab109250	RRID: AB_10863442
	Rabbit anti-SOX2	1:100	Abcam Cat#ab97959	RRID: AB_2341193
	Mouse anti-SSEA4	1:100	Abcam Cat#ab16287	RRID: AB_778073
	PE Mouse anti-Human Tra-1–60	1:100	BD Pharmingen Cat#560193	RRID: AB_1645539
	Alexa 647 Mouse anti-SSEA-4	1:200	BD Pharmingen Cat#560796	RRID: AB_2033991
Germ layer markers	Rabbit anti-PAX6	1:200	Life technologies Cat#426600	RRID: AB_2533534
	Rabbit anti-FOXA2	1:250	Life technologies Cat#701698	RRID: AB_2576439
	Rabbit anti-TBX6	1:200	Life technologies Cat#PA535102	RRID: AB_2552412
Secondary antibodies	Alexa 488 goat anti-mouse IgG	1:500	Life technologies Cat#A11001	RRID: AB_2534069
	Alexa 594 donkey anti-rabbit IgG	1:500	Life technologies Cat#A21207	RRID: AB_141637
Isotype control	PE Mouse IgG1 K	1:100	BD Pharmingen Cat#555749	RRID: AB_396091
	Alexa 647 Mouse IgG1 k	1:100	BD Pharmingen Cat#557783	RRID: AB_396871

	Primers		
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**
Pluripotency markers (qRT-PCR)		N/A	Applied Biosystems
	POU5F1 (OCT4)		Hs04260367_gH_FAM
	SOX2		Hs01053049_s1_FAM
	NANOG		Hs02387400_g1_FAM
House-Keeping Genes (qRT-PCR)		N/A	Applied Biosystems
	GAPDH		Hs02758991_g1_VIC
Three germ layer marker (qRT-PCR)		N/A	Applied Biosystems
	PAX6		Hs01088114_m1_FAM
	NESTIN		Hs04187831_g1_FAM
	BRACHYURY		Hs00610080_m1_FAM
	GSC		Hs00418279_m1_FAM
	SOX17		Hs00751752_s1_FAM
	AFP		Hs01040598_m1_FAM
Targeted mutation analysis/ sequencing	ABCG2 exon 4	400 bp	Forward: AGCATGTGTTGGAGGGAAAA Reverse: TGTCCTTACAAGGGCCA TATTC

4.8. STR analysis

STR analysis of 16 specific loci for the YUCMi020-A and corresponding PBMCs was performed by Macrogen, Inc.

4.9. ABCG2 gene sequencing

Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen), following the manufacturer's protocols. Direct sequencing of biallelic mutations in exon 4 of ABCG2 gene, including c.376 position, was carried out by Macrogen, Inc. The primers used in this study are listed in Table 1.

CRediT authorship contribution statement

Youn Keong Cho: Writing – review & editing, Writing – original draft, Investigation. **Hyun-Kyung Kim:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Eun Jung Suh:** M, ethodology. **Hyun Ok Kim:** Supervision, Conceptualization. **Sinyoung Kim:** Supervision, Project administration, Funding acquisition.

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.

Data availability

STR analysis data is archived with journal.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2024.103434.

References

Cho, Y.K., Kim, H.K., Kwon, S.S., Jeon, S.H., Cheong, J.W., Nam, K.T., Kim, H.S., Kim, S., Kim, H.O., 2023. In vitro erythrocyte production using human-induced pluripotent stem cells: Determining the best hematopoietic stem cell sources. Stem Cell Res. Ther. 14, 106.

Kim, M.S., Kim, J.S., Park, H., Chung, Y., Kim, H., Ko, D.H., Hwang, S.H., Won, H.S., Oh, H.B., 2020. Fatal hemolytic disease of the fetus and newborn caused by anti-Jr (a) antibody: A case report and literature review. Transfus Apher. Sci. 59, 102605.

Toshimitsu, M., Nagaoka, S., Kobori, S., Takahashi, Y., Murotsuki, J., 2019. Successful management of the fetal severe anemia associated with Jra alloimmunization by intrauterine transfusion of Jr(a+) red blood cells. Case Rep. Obstet. Gynecol. 2019, 5174989.