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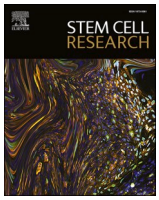

Lab Resource: Multiple Cell Lines

Generation of two human induced pluripotent stem cell lines from peripheral blood mononuclear cells of clozapine-tolerant and clozapine-induced myocarditis patients with treatment-resistant schizophrenia

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A B S T R A C T

Clozapine has superior efficacy in the treatment of refractory schizophrenia; however, use of clozapine is limited due to severe side effects, including myocarditis. Using non-integrative Sendai virus, we generated induced pluripotent stem cell lines from peripheral blood mononuclear cells of two patients with refractory schizophrenia, one clozapine-tolerant and one clozapine-induced myocarditis. Both cell lines exhibited a normal karyotype and pluripotency was validated by flow cytometry, immunofluorescence and their ability to differentiate into the three germ layers. These lines can be used to generate 2D and 3D patient-specific human cellular models to identify the mechanism by which clozapine induces myocardial inflammation.

1. Resource table

Unique stem cell lines identifier	UOCi001-A UOCi002-A
Alternative name(s) of stem cell lines	*CIM001.4* (UOCi001-A) *CIM008.6* (UOCi002-A)
Institution	University of Calgary
Contact information of distributor	Dr. Chad Bousman, chad.bousman@ucalgary.ca
Type of cell lines	Induced pluripotent stem cells (iPSCs)
Origin	Human

(*continued on next column*)

(*continued*)

Additional origin info required *for human ESC or iPSC*	UOCi001-A Age: 36 Sex: F Ethnicity: Southeast Asian UOCi002-A Age: 34 Sex: M Ethnicity: Southeast Asian
Cell Source	Peripheral blood mononuclear cells
Clonality	Clonal
Method of reprogramming	

(*continued on next page*)

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(*continued*)

	Non-integrative Sendai virus delivery of OCT4, SOX2, KLF4 and c-MYC transgenes
Genetic Modification	No
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	N/A
Associated disease	Schizophrenia, Clozapine-induced myocarditis
Gene/locus	N/A
Date archived/stock date	01 June 2017
Cell line repository/bank	UOCi001-A https://hpscreg.eu/cell-line/UOCi001-AUOCi002-A https://hpscreg.eu/cell-line/UOCi002-A
Ethical approval	Conjoint Health Research Ethics Board at the University of Calgary (Reference number: REB17-1994) and Melbourne Health Human Research Ethics Committee (Reference number: 2017.007).

2. Resource utility

The mechanism by which clozapine induces myocardial inflammation and injury is unknown. These Induced pluripotent stem cell (iPSC) lines will serve as a crucial resource for identifying the mechanism as well as facilitate future screening of novel or repurposed drug candidates for the treatment of schizophrenia.

3. Resource details

Schizophrenia is a complex and heterogeneous syndrome that affects 1 % of the world's population (McGrath et al., 2008). Antipsychotic medications relieve symptoms, prevent disease progression, and reduce disease-related morbidity and mortality (Lieberman et al., 2001). Unfortunately, 30–60 % of patients with schizophrenia show incomplete resolution of their symptoms despite trials of several antipsychotic medications and are defined as 'treatment-resistant schizophrenia' (TRS) (Howes et al., 2017). Clozapine is the most effective and only FDA-approved drug for TRS. However, use of clozapine is limited due to severe idiosyncratic adverse drug reactions, including myocarditis. To determine the cellular mechanism by which clozapine induces myocardial inflammation and to identify candidate pharmacotherapies to prevent and/or treat clozapine-induced myocarditis, we generated iPSC lines from TRS patients with and without a history of clozapine-induced myocarditis.

Two TRS patients were recruited by investigators affiliated with the Pharmacogenomics of Clozapine-Induced Myocarditis (PROCLAIM) Consortium (Bousman, et al., 2018). Individuals were eligible for inclusion into the study if they: (a) were aged 18 to 65 years, (b) had a clinical diagnosis of a schizophrenia-spectrum disorder, (c) had accessible medical records, and (d) had a history of clozapine therapy. For cases, an additional inclusion criterion was a history of myocarditis evidenced by abnormal troponin I/T levels (>2 times upper limit of normal) or signs of left ventricular dysfunction with or without elevated C-reactive protein levels (>100 mg/L) during the first 30 days of clozapine therapy, which aligns with current monitoring guidelines (Ronaldson et al., 2011). Importantly, cases with an infection at the onset of clozapine-induced myocarditis were excluded as part of the differential diagnosis of myocarditis. An additional inclusion criterion for clozapine-tolerant controls was evidence of clozapine therapy for a minimum of 45 days with no documented history or clinical suspicion of myocarditis.

Following institutional ethical committee approval, a whole blood sample was taken from each patient. Human iPSC lines were generated by isolation of the patient's peripheral blood mononuclear cells (PBMCs) and transduced with a non-integrative Sendai virus containing the reprogramming transgenes, OCT4, SOX2, KLF4 and c-MYC. After 21 days of reprogramming, iPSC colonies were selected for further expansion and characterization. Both iPSC lines displayed normal stem cell morphology, characterized by small and tightly packed cells, high nucleus/cytoplasm ratio, and prominent nucleoli (Fig. 1A). The pluripotency of the cells was qualitatively confirmed by immunofluorescence staining using the pluripotency marker SSEA4 and OCT4 (Fig. 1B). In addition, flow cytometry confirmed that cell lines strongly expressed pluripotency markers TRA181, SSEA4, CD9 and EPCAM (Fig. 1C).

Both iPSC lines formed embryoid bodies in vitro and were able to develop three germ layers. Immunofluorescence staining confirmed expression of the endodermal marker (α-Fetoprotein), ectoderm marker (β-Tubulin) and the mesodermal marker (smooth muscle actin) (Fig. 1D). Both iPSC lines were subjected to karyotype analysis and showed a normal karyotype (with 0.5 Mb resolution). Concordance analysis of SNP array data from PBMC samples and iPSC lines confirmed that the two samples were from the same individuals (Table 1). Both lines were confirmed to be free from mycoplasma contamination (Supplementary Material). Table 1 summarizes all characterization and validation analyses of the iPSC lines.

4. Materials and methods

4.1. Isolating PBMCs from patient blood samples

PBMCs isolation was performed by diluting the whole blood with an equal volume of PBMC dilution buffer and layered over Lymphoprep (Stem Cell Tech, cat#07801) in SepMate-15 tubes (StemCell Technologies). Samples were centrifuged at 1200 rcf for 10 min at room temperature, transferred to a 15 ml tube, washed with PBMC dilution and wash buffer and centrifuged at 300 rcf for 10 min. After isolation, 5×10^5 PBMCs were cultured in Erythroid expansion medium (Stem Cell Tech; Cat# 09,605 and 02692) for 7 days, with media replenished every other day.

4.2. Reprogramming the PBMCs to iPSCs

PBMCs were reprogrammed using erythroid expansion medium + Sendai virus (Life Tech; Cat# A167517) with KOS MOI (5), hc-Myc MOI (5), and h-Klf4 MOI (5). Transduced cells were plated onto mouse embryonic fibroblasts (MEFs) in a mix of StemSpan SFEM II (Stem Cell Tech; Cat #09605) and iPSC media. iPSC colonies were mechanically isolated and passaged onto MEFs until the lines were stable. The lines were adapted to feeder free culture on Vitronectin (Stemcell technology) coated plate in Matrigel/ Essential 8 medium (ThermoFisher Scientific) for continued culture. All cells were maintained at 37∘C with 10 % Co2.

4.3. Pluripotency marker Immunocytochemistry

The iPSCs (passage 6) were fixed in 4 % paraformaldehyde for 15 min and permeabilization with 0.1 % TritonX-100 in PBS for 15 min at room temperature. Non-specific binding was blocked with 10 % Bovine Serum Albumin (BSA) for 30 min at room temperature. Cells were incubated with primary antibodies for 3 h at 4 ∘C, followed by secondary antibodies for 1hr at room temperature (Table −2). Cell nuclei were next stained with 1ug/ml DAPI and imaged with a ImageXpress microscope using a 20X objective.

4.4. Flow cytometry analysis

Harvested iPSCs (passage 3) were filtered through a cell-strainer cap to a fluorescence-activated cell sorting tube. Cells were then incubated with conjugated antibodies TRA-1–60, SSEA4, and CD9 for 15 min on ice (Table 2). Samples were analyzed using LSRFortessa (BD Bioscience) BD FACSDiva and FCS Express software.

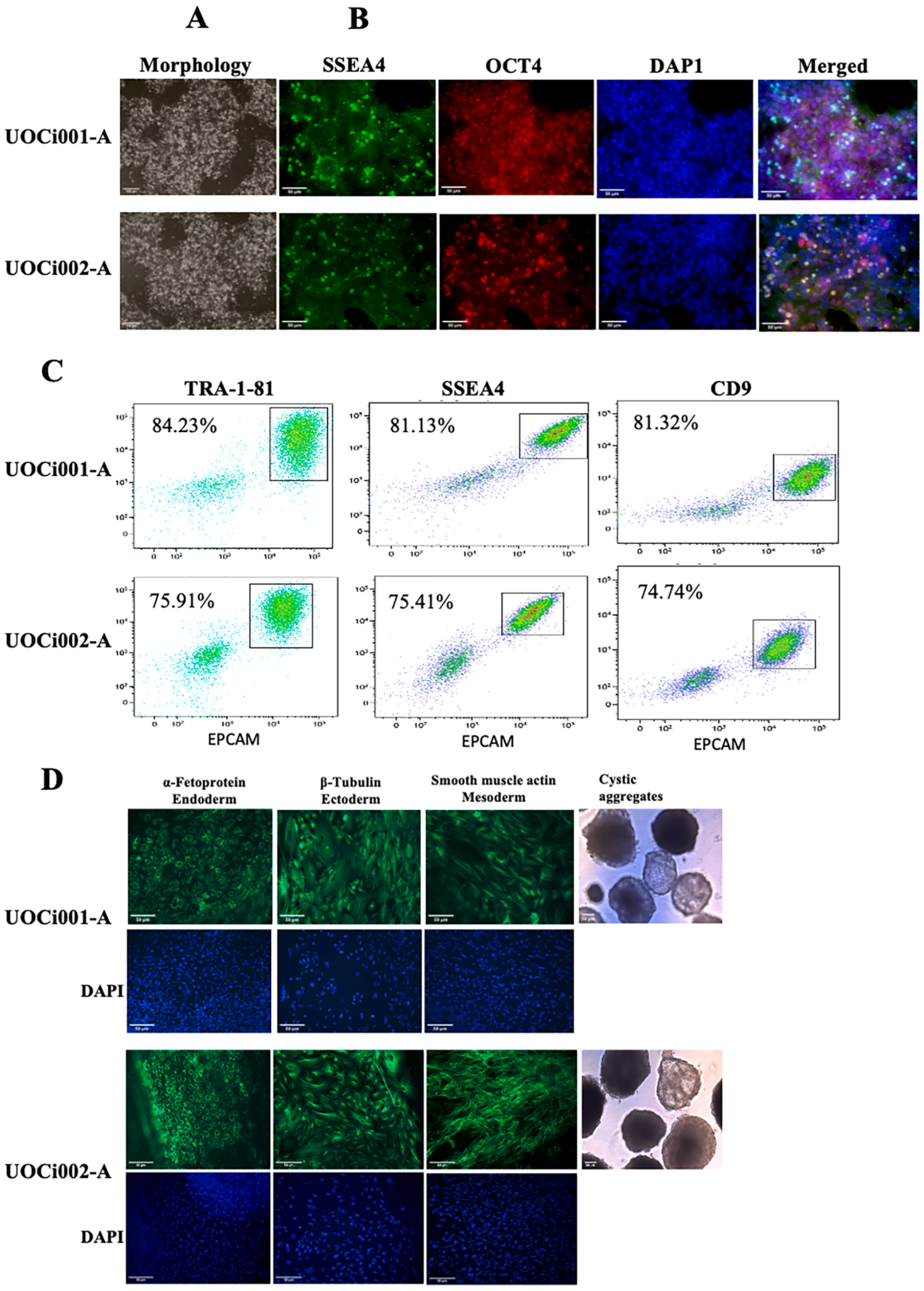

Fig. 1. Molecular and cellular characterization of iPSC lines.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1, panel A
Phenotype	Qualitative analysis Immunocytochemistry staining	Positive staining for pluripotency markers: OCT4 and SSEA4	Fig. 1, panel B
	Quantitative analysis Flow cytometry	TRA-1–81:>75.91 % SSEA4: >75.41 % CD9: >74.74 %	Fig. 1, panel C
Genotype	Karyotype (G-banding) and resolution	UOCi001-A: Normal karyotype 46, XX, (0.50 Mb)UOCi002-A: Normal karyotype 46, XY, (0.50 Mb)	Supplementary Material
Identity	Microsatellite PCR (mPCR) OR	N/A	N/A
	STR analysis	Concordance analysis was performed on 1,933,117 sites on the Infinium Global Diversity Array with Enhanced PGx. Concordance was >99.8 %.	Not shown but available with author
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by qPCR. Negative	Not shown but available with author
Differentiation potential	Embryoid body formation (immunofluorescence)	Expression of ectoderm markers (β-Tubulin), endoderm markers (α-Fetoprotein) and mesoderm markers (SMA)	Fig. 1, panel D
List of recommended germ layer markers	Expression of protein levels (IF)	Ectoderm: TUBB3 Endoderm: AFP Mesoderm: SMA	Immunofluorescence
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
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	Rabbit anti-OCT4 monoclonal antibody	1:100	ThermoFisher Scientific Cat#A24867	RRID: AB_2650999
	Mouse anti-SSEA4 monoclonal antibody	1:100	ThermoFisher Scientific Cat#A24866	RRID: AB_2650999
	Alexa Fluor 647 anti-human TRA-1-81 antibody	1:100	BioLegend Cat# 330706	RRID: AB_1089242
	PE conjugated anti-human CD326 (EPCAM) antibody, Clone EBA-1	1:30	BD Biosciences Cat# 347198	RRID: AB_400262
	PE/Cy7 anti-human SSEA-4 antibody	1:100	BioLegend Cat# 330420	RRID: AB_2629631
	Mouse Anti- CD9 Monoclonal Antibody, FITC Conjugated, Clone M-L13	1:10	BD Biosciences Cat# 555371	RRID: AB_395773
Differentiation Markers	Mouse Anti-Actin, α-Smooth Muscle antibody	1:2000	Sigma-Aldrich Cat# A2547	RRID: AB_476701RRID: AB_258392
	Mouse Anti-α-Fetoprotein (AFP) antibody	1:2000	Sigma-Aldrich Cat# A8452	RRID: AB_258392
	Anti β-Tubulin antibody	1:2000	Sigma-Aldrich Cat# T8578	RRID: AB_1841228
Secondary antibodies	Donkey anti-rabbit Alexa Fluor™ 594	1:250	ThermoFisher Scientific Cat#A24870	RRID: AB_2650999
	Goat anti-mouse Alexa Fluor™ 488	1:250	ThermoFisher Scientific Cat#A24877	RRID: AB_2650999
	Alexa Fluor 488 goat anti-mouse antibody	1:1000	ThermoFisher Scientific Cat# A11029	RRID: AB_2534088

4.5. Germ layer directed differentiation

Cells (passage 7) were cultured in differentiation medium consisting of DMEM, 18 % FBS, 1 mM glutamine, 0.1 mM-mercaptoethanol and 0.1 mM non-essential amino acids. At day 14, immunofluorescence staining of embryoid bodies (EBs) was performed to detect three germ layers using antibodies against smooth muscle actin (mesoderm), α-Fetoprotein (endoderm), and β-Tubulin (ectoderm). After three washes, secondary antibody Alexa Fluor 488 goat anti-mouse antibody was used. Cells were imaged with OLYMPUS IX70 microscope using a 20X objective.

4.6. Karyotyping and SNP analysis

Both iPSC lines (Passage 3) were karyotyped by the Victorian Clinical Genetics Services at Murdoch Children's Research Institute (Parkville, Australia), using Illumina Infinium GSA-24 v1.0 Genotyping array at a resolution of 0.50 Mb. The data was compared to the human reference sequence UCSC GRCh37/hg19 human reference sequence. Comparison of PBMC and iPSC lines using 1,933,117 sites on the Infinium Global Diversity Array were performed using a SNP concordance analysis.

4.7. Mycoplasma testing

Mycoplasma contamination was assessed in the iPSC lines (passage 3) by PCR (Cerberus Sciences, Adelaide, Australia).

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Nazanin Vaziri reports financial support was provided by Natural

Sciences and Engineering Research Council of Canada. Chad Bousman reports a relationship with Sequence2Script Inc that includes: equity or stocks.

Data availability

Data will be made available on request.

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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.2022.102877.

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