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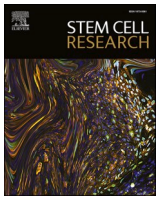

Lab Resource: Single Cell Line

Generation of a human induced pluripotent stem cell line (UMGACBi001-A) from urine cells of a chronic kidney disease patient with hypertension, diabetic nephropathy and acute sepsis

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A B S T R A C T

Chronic kidney disease is a major public health burden associated with a drastically reduced quality of living and life span that lacks suitable, individualized therapeutic strategies. Here we present a human induced pluripotent stem cell line (iPSC, UMGACBi001-A) reprogrammed from urine cells of an acute septic dialysis patient suffering from chronic kidney disease using non-integrating administration of RNAs. The generated iPSCs were positively characterized for typical morphology, pluripotency marker expression, directed differentiation potential, non-contamination, chromosomal consistency and donor identity. This iPSC-line can be a useful source for *in vitro* disease modelling and individualized therapeutic approaches.

1. Resource table

Unique stem cell line identifier	UMGACBi001-A
Alternative name(s) of stem cell line	iPSC 1.3.3 DUSC-CKD
Institution	Institute for Anatomy and Cell Biology, University Medicine Greifswald, Greifswald, Germany
Contact information of distributor	Dr. rer. nat. Tim Lange, tim.lange@uni-greifswald.de
Type of cell line	iPSC
Origin	human
Additional origin info required for human ESC or iPSC	Age: 71 Sex: male Ethnicity if known: European
Cell Source	Urine cells
Clonality	Clonal
Method of reprogramming	Non-integrating mRNA/miRNA
Genetic Modification	N/A
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	N/A
Associated disease	Septic acute on chronic renal failure with underlying hypertension and diabetic nephropathy receiving temporal dialysis
Gene/locus	N/A
Date archived/stock date	2023
Cell line repository/bank	Human Pluripotent Stem Cell Registry (hPSCreg®) https://hpscreg.eu/user/cellline/edit/UMGACBi001-A
Ethical approval	This work was approved by the Ethics Commission of the University Medicine Greifswald- BB 166/19

2. Resource utility

The present iPSC line UMGACBi001-A was derived from urine cells of a 71 years old male donor suffering from chronic kidney disease (CKD). This iPSC line can be used for individualized disease-modelling,

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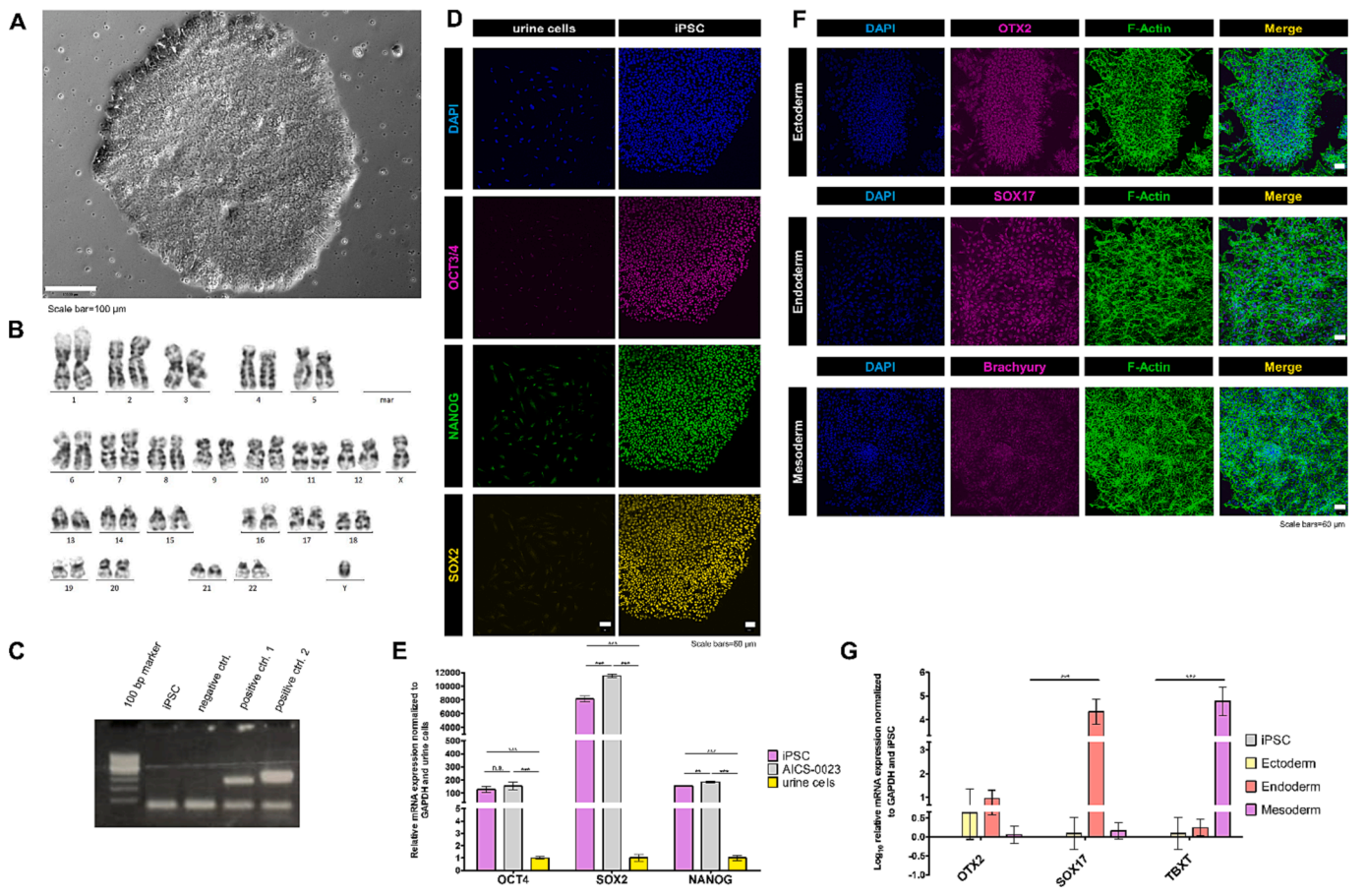

Fig. 1.

treatment development or drug screening and for differentiation towards specific cell types or whole organoids.

3. Resource details

Cultured urine sediment cells were reprogrammed with the Stemgent® StemRNA™ 3rd Gen Reprogramming Kit for Reprogramming Urine Derived Progenitor Cells (REPROCELL Europe Ltd., Durham, UK). Reprogramming was based on integration-free transfections of RNAs (OCT4, SOX2, cMYC, LIN28, KLF4, NANOG, E3, K3, B18 and miR-302/367-cluster) and was performed under feeder-free conditions. The derived cell line was isolated from a single colony positive for TRA-1-60 and a typical iPSC morphology. The cell line appears as undifferentiated colonies with sharply defined borders and hardly distinguishable single cells with a high nucleus-to-cell body-ratio and prominent nucleoli (Fig. 1A) (Han et al., 2022). The generated cell line exhibits a normal karyotype of 46XY (Fig. 1B) and is free of mycoplasma contamination as detected by RT-PCR (Fig. 1C) and lateral flow test (data not shown). Donor testing for HIV1 + 2, HPVB and HPVC was negative and STR-analysis showed a perfect match for all 21 gene loci (STR-analysis). Immunofluorescence staining (Fig. 1D) and RT-qPCR (Fig. 1E) revealed an overexpression of pluripotency markers OCT3/4, SOX2 and NANOG that is comparable with a commercially available iPSC line (AICS-0023) (Pilz et al., 2022). Pluripotency was further analysed by directed differentiation using the Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems, Inc., Minneapolis, MN, USA) and RT-qPCR. Cells were seeded at passage 11 and treated with the corresponding differentiation media for 3 days (ectoderm and endoderm) and for 2 days (mesoderm). Immunofluorescence staining showed an expression of ectoderm marker OTX2, endoderm marker SOX17 and mesoderm marker BRACHYURY in directly differentiated cells (Fig. 1F). Quantification of the same markers by RT-qPCR confirmed these observations (Fig. 1G) (Table 1).

4. Materials and methods

Urine cells were isolated and cultured as described before (Zhou et al., 2012). After adhering successfully, the culture media was switched to MesenCult™ Proliferation Kit (Human, STEMCELL Technologies, Vancouver, B.C., Canada) to trigger proliferation. Reprogramming was performed in one well of a 6-well plate coated with 2.4 µg/mL iMatrix-511 substrate (Takara Bio Europe SAS, Saint-Germain-en-Laye, France) diluted in PBS. Starting cell count was $7.5x10^4$ cells/well. Reprogramming was performed with the Stemgent® StemRNA™ 3rd Gen Reprogramming Kit for Reprogramming Urine Derived Progenitor Cells (REPROCELL Europe Ltd) after manufacturer's instructions. The cells were cultured in reprogramming media and were transfected daily, seven times in a row. Between daily media exchange and the following transfection, 8 h were left out to protect cells from too much transfection stress. After 7 days media was switched to NutriStem hPSC XF media (Sartorius AG, Göttingen, Germany) without antibiotics and was changed daily. After 13 days colonies were screened for typical iPSC morphology and TRA-1-60 positivity and picked with a cannula. Picked colonies were transferred to new iMatrix-511-coated plates and were maintained in mTeSR™1 media (STEMCELL Technologies) plus 100 U/mL penicillin and 0.1 mg/mL streptomycin (Thermo Fisher Scientific, Waltham, MA, USA) at 37 °C and 5 % CO_2. Up from passage 4 cells were passaged weekly using ReLeSR™ (STEMCELL Technologies) in a 1:8 split-ratio and cryo-conserved using mFreSR™ (STEMCELL Technologies). Karyotyping and STR-analysis were performed at

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	normal	Fig. 1 panel A
Phenotype	Qualitative analysis Immunocytochemistry	OCT3/4, NANOG, SOX2	Fig. 1 panel D
	Quantitative analysis RT-qPCR	OCT4: 210.2-fold NANOG: 188.4-fold SOX2: 4907.1-fold	Fig. 1 panel E
Genotype	Karyotype (G-banding) and resolution	46XY, Resolution 400	Fig. 1 panel B
Identity	Microsatellite PCR (mPCR) OR STR analysis	N/A 21 sites, all matched	N/A STR-Analysis table
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	RT-PCR and lateral flow Negative	Fig. 1 C
Differentiation potential	Directed differentiation	Ectoderm Endoderm Mesoderm	Fig. 1 panel F and G
List of recommended germ layer markers	**Expression of germ layer markers was tested at mRNA (RT-qPCR) and protein (IF) level.**	Positive expression of: Ectoderm: OTX2, Endoderm: SOX17 Mesoderm: BRACHYURY proven by RT-qPCR and IF	RT-qPCR with reference gene GAPDH normalized against iPSCs (Fig. 1 G) IF with specific antibodies (Fig. 1 F)
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative	not shown but available with author
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

passage 8 as previously described (Zywitza et al., 2022; Lässig et al., 2021). For mycoplasma testing we used the PCR Mycoplasma Test Kit (AppliChem GmbH, Darmstadt, Germany) and the MycoStrip™ - Mycoplasma Detection Kit (InvivoGen, Toulouse, France) regularly. For directed differentiation we used the Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems) after manufacturer's instructions. Immunofluorescence staining for pluripotency markers was performed with the Human Pluripotent Stem Cell 3-Color Immunocytochemistry Kit (R&D Systems) and for directed differentiation marker detection we used the Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems) after manufacturer's instructions. Prior to this, cells were plated on iMatrix-511 coated plastic cover slips and were cultured for 5 days. Afterwards cells were fixed for 10 min in 2 % PFA and blocked (PBS, 2 % fetal bovine serum, 2 % bovine serum fraction V, 0.2 % fish gelatine) for 45 min. Nuclei were counterstained with 1 μg/mL DAPI (Sigma-Aldrich, St. Louis, MO, USA) in PBS for 5 min. Cover slips were mounted in Mowiol (Carl Roth, Karlsruhe, Germany). Confocal laser scanning microscopy was performed using an Olympus FV3000 system (EVIDENT Europe GmbH, Hamburg, Germany) equipped with 405, 488, 561 and 640 nm laser lines. For imaging a 20×, 0.8NA air objective was used. RNA isolation, reverse transcription and RT-qPCR were performed as previously described (Ristov et al., 2022). For RT-qPCR we used QuantStudio3 (Thermo Fisher Scientific, Darmstadt, Germany) with the following cycling scheme: initial 10 min 95 °C, followed by 40 cycles of 15 sec 95 °C, 20 sec 60 °C, 20 sec 72 °C followed by 15 sec 95 °C. We also included a melting curve analysis with the following scheme: 1 min 60 °C followed by ramping to 95 °C with 10 data points per degree. No-template and no-reverse-transcriptase controls were included (Table 2).

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers	NL557-Goat Anti-Human SOX2 NL637-Goat Anti-Human OCT-3/4 NL493-Goat Anti-Human NANOG	1:100	R&D #SC021 Human Pluripotent Stem Cell 3-ColorImmunocytochemistry Kit	AB_355110 AB_354975 AB_2235855
Differentiation Markers	Goat anti-human SOX17 Goat anti-human OTX2 Goat anti-human BRACHYURY	1:50	R&D #SC027B Human Pluripotent Stem Cell Functional Identification Kit	AB_355060 AB_2157172 AB_2200235
Secondary antibodies	ATTO-555-donkey anti-goat	1:300	Invitrogen #A-21432	AB_2535853
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Pluripotency Marker	OCT4(Han et al., 2022)	164 bp	for: CCTCACTTCACTGCACTGTA rev: CAGGTTTTCTTTCCCTAGCT	
Pluripotency Marker	SOX2(Han et al., 2022)	151 bp	for: CCCAGCAGACTTCACATGT rev: CCTCCCATTTCCCTCGTTTT	
Pluripotency Marker	NANOG(Han et al., 2022)	237 bp	for: AAGGTCCCGGTCAAGAAACAG rev: CTTCTGCGTCACACCATTGC	
House-keeping gene	GAPDH(Han et al., 2022)	153 bp	for: GTGGACCTGACCTGCCGTCT rev: GGAGGAGTGGGTGTCGCTGT	
Ectoderm marker	OTX2	177 bp	for: ACACAGCCTCCACTGTGATT rev: GGTGGACAGGTTCAGAGTCC	
Endoderm marker	SOX17	296 bp	for: GACATGAAGGTGAAGGGCGA rev: GGCCGGTACTTGTAGTTGGG	
Mesoderm marker	TBTX	294 bp	for: GCTCACCAACAAGCTCAACG rev: CCTGATTGGGAGTACCCAGG	

RRID Requirement for antibodies: use https://antibodyregistry.org/ to retrieve RRID for antibodies and include ID in table as shown in examples.

5. Cell line identity testing

Identical

6. Abnormal karyotype

No

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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.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2023.103224.

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