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Lab resource: Stem cell line

Generation of a human induced pluripotent stem cell line from urinary cells of a healthy donor using integration free Sendai technology

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ABSTRACT

We have generated a human induced pluripotent stem cell (iPSC) line derived from urinary cells of 1 28–30 year old healthy female donor. The cells were reprogrammed using a non-integrating viral vector and shown to have full differentiation potential. Together with the iPSC-lines, the donors provided blood cells for the study of immunological effects of the iPSC line and its derivatives in autologous and allogeneic settings. The line is available and registered in the human pluripotent stem cell registry as BCRTi004-A.

1. Resource Table

Name of Stem Cell construct	BCRTi004-A
Institution	Charité—Universitätsmedizin Berlin, Berlin Brandenburg Center for Regenerative Therapies (BCRT)
Person who created resource	Bella Rossbach
Contact person and email	Andreas Kurtz (Andreas.Kurtz@charite.de)
Date archived/stock date	February, 2015
Origin	Cells isolated from human urine
Type of resource	Human induced pluripotent stem cells (hiPSC) derived from a healthy female donor
Sub-type	hiPSC line
Key transcription factors	OCT4, SOX2, MYC, KLF4
Authentication	STR Analysis/Fingerprinting (available in hPSCreg under controlled access)
Link to related literature (direct URL links and full references)	
Information in public databases	http://hpscreg.eu

(See Figs. 1– 4.)

2. Material and methods

2.1. Ethical statement

This work was approved by the Ethics Commission of the Charité—Universitätsmedizin Berlin (EA4/110/10).

2.2. Urinary cell isolation and expansion

Urine was obtained from a female healthy Caucasian donor of 28–30 years of age. Urinary cells were isolated according to the protocol described by Zhou et al., 11 (Zhou et al., 2012). After centrifugation and washing with PBS (Gibco) the obtained cell pellet was resuspended in primary-medium containing DMEM high glycose (GIBCO) and HAM's F12 (Biochrom) which were mixed in a 1:1 ratio and supplemented with 2.5 μg/ml amphotericin B (Biochrom), 10% (*v*/*v*) fetal bovine serum (FBS) (Biochrom), penicillin/streptomycin (P/S, 100 U/ml/100 μg/ml, Biochrom) and the renal growth media (REGM) SingleQuot kit (Lonza) and finally plated on 0.1% w/t gelatin coated (Milipore) plates. Primary-medium was added for the following three days and afterwards successively changed to proliferation-medium which was a 1:1 mixture of DMEM high glucose and the REBM basal media supplemented with 5% (*v*/*v*) FBS, 1,25 mM GlutaMax™, 0,5% (*v*/*v*) non-essential amino acids (NEAA, Gibco), P/S (50 U/ml, 50 μg/ml), 2,5 ng/ml basic fibroblast growth factor (bFGF), 2.5 ng/ml platelet derived growth factor (PDGF-AB), 2.5 ng/ml human epidermal growth factor (hEGF, all Peprotech)

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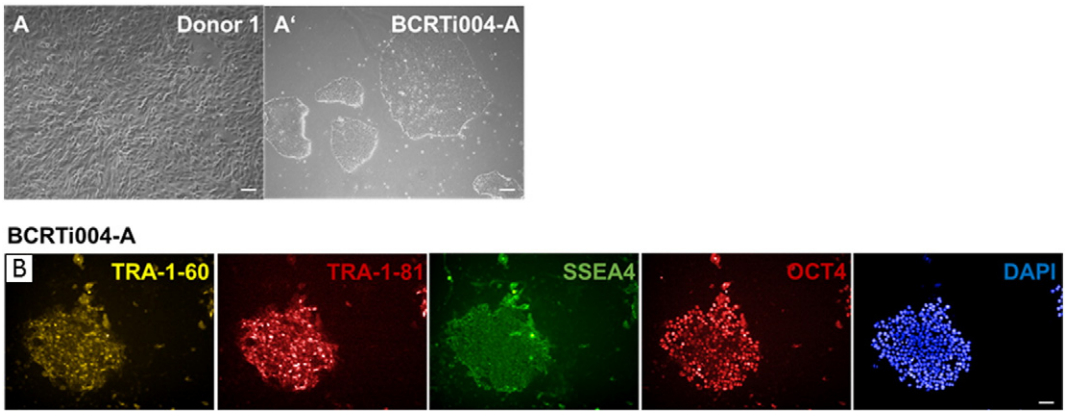

Fig. 1. Morphology of primary urinary cells, reprogrammed hiPSCs and pluripotency marker expression. (A) Phase contrast images of primary urinary cells and (A′) generated hiPSCs. (B) hiPSC were stained for the pluripotency markers TRA-1-60, TRA-1-81, SSEA-4, OCT4 and DAPI for staining of nuclei. Scale bars: A, A′: 100 μm, B: 50 μm.

and the REGM SingleQuot kit. First colonies appeared on day 5. After reaching about 90% confluency, cells were passaged using trypsin (Biochrom) and re-plated on gelatin coated dishes in a ratio 1:10. Cells were frozen in FBS containing 10% dimethylsulfoxide (DMSO).

2.3. Reprogramming and hiPSC maintenance

Urinary cells were thawed and cultivated in RPMI (Gibco) medium supplemented with 10% FBS and 1% P/S on gelatin coated dishes until reaching confluence of around 90% again. The Sendai virus (SeV) based non-integration CytoTune™—iPS Reprogramming Kit (Life Technologies) (Fusaki et al., 2009) was used according to manufacturer's instructions. First colonies appeared after 10 days. 5 single colonies were picked and cultivated under feeder free conditions on Geltrex (Life Technologies) in TeSR-E8 media (Stemcell Technologies) supplemented with 1% P/S. The hiPSCs were passaged every 5–7 days in a ratio 1:6. At passage 14, colonies were tested for virus loss and pluripotency marker expression and one of the 5 colonies was kept in culture for further analysis. A frozen stock of the other colonies, which were also reprogrammed, was kept.

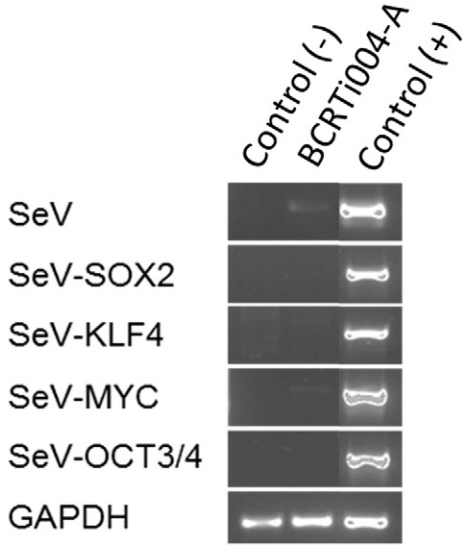

Fig. 2. Analysis of Sendai viral transgene expression in generated hiPSC lines. RT-PCR of reprogrammed hiPSC lines was performed for the expression of Sendai virus (SeV), SeV-SOX2, SeV-KLF4, SeV-MYC and SeV-OCT3/4.

2.4. Pluripotency marker staining of hiPSCs

hiPSCs were fixed (BD Cytofix) and permeabilized using Perm/Wash buffer (BD) and stained with antibodies against the pluripotency markers TRA-1-60 (Novous Biologicals), TRA-1-81 (Novus Biologicals), OCT-4 PerCP-Cy5.5 (BD) and SSEA-4 FITC (R&D). Subsequently, cell nuclei were labeled with 4′,6-Diamidin-2-phenylindo (DAPI, Invitrogen). The final analysis was performed using the High Content Screening Imaging System Operetta (Perkin Elmer).

2.5. RNA isolation and cDNA synthesis

Total RNA was extracted (Qiagen RNA Mini Kit) and transcribed into cDNA using the SuperScript® III Transcriptase Kit (Life Technologies).

2.6. Detection of SeV genome and transgenes

After 14 passages, hiPSC lines were tested for SeV residues. PCR was performed using primers and instructions (Table 1) as recommended by the manufacturer. As positive control RNA was used from the reprogramming leftovers. Negative control RNA was obtained from the hiPSC line WISCi004-A (IMR90, WiCell), which was lentivirally reprogrammed from IRM90.4 fibroblasts.

2.7. Embryoid body (EB) formation and germ layer differentiation

To test the capacity of the reprogammed hiPSC line to spontaneously differentiate into cells of all three germ layers, hiPSCs were harvested using accutase (Gibco) and seeded in TeSR-E8 supplemented with

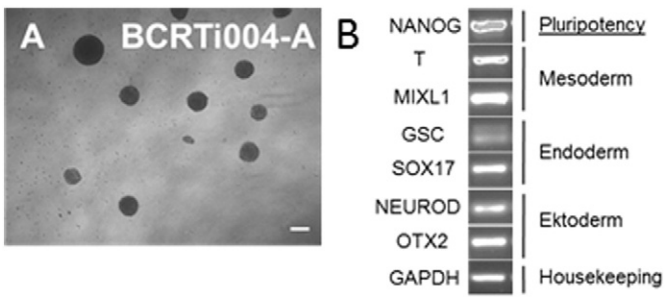

Fig. 3. Spontaneous *in vitro* differentiation of hiPSCs into cells of the three germ layers. (A) EB formation and (B) RT-PCR for the markers NANOG, Brachyury (T), Mix Paired-Like Homeobox (MIXL1), Goosecoid (GSC), SRY (Sex Determining Region Y)-Box 17 (Sox17), NeuroD, Orthodenticle homeobox 2 (OTX2) and for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Scale bar: 100 μm.