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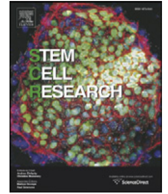

Lab Resource: Stem Cell Line

Characterization of dermal fibroblast-derived iPSCs from a patient with high grade steatosis

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ABSTRACT

Primary fibroblasts from a high grade steatosis patient were reprogrammed by transduction of retroviruses OCT4, SOX2, c-MYC and KLF4. IPSCs were characterized by immunocytochemistry, embryoid body-formation, DNA-fingerprint, karyotype analysis and comparative transcriptome analyses with the human embryonic stem cell line H1 revealed a Pearsons correlation coefficient of 0.9287.

Resource table.

Name of stem cell construct	S08
Institution	Institute for Stem Cell Research and Regenerative Medicine
Person who created resource	Justyna Jozefczuk
Contact person and email	James Adjaye, James.Adjaye@med.uni-duesseldorf.de
Date archived/stock date	August 2012
Origin	Primary human fibroblasts
Type of resource	Biological reagent: induced pluripotent stem cell (iPSC); derived from steatosis patients and a healthy individual
Sub-type	Cell line
Key transcription factors	OCT4, SOX2, c-MYC and KLF4
Authentication	Identity and purity of cell line confirmed (Figs. 1, 2)
Link to related literature (direct URL links and full references)	http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4672680/
Information in public databases	None

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1. Resource details

Primary fibroblasts from steatosis patient H0008 (male, 61 years, high grade -steatosis) were reprogrammed by transduction of retroviruses encoding for OCT4, SOX2, KLF-4 and c-MYC (H0008; Jozefczuk et al., 2012). The pluripotency-associated transcription factors OCT4, NANOG, SOX2 and cell surface markers SSEA-4, TRA-1-60, TRA-1-81 were expressed. Pluripotency was further demonstrated *in vitro* by embryoid body (EB)-based differentiation to the three germ layers endoderm, ectoderm, and mesoderm. DNA fingerprinting confirmed the origin of the iPSC line, while karyogram revealed a 46,XY karyotype.

2. Materials and methods

2.1. Patient recruitment, sample collection and clinical measurements

The study was approved by the institutional review board of the Medical University of Graz (reg. IRB00002556 at the Office for Human Research Protections of the US Departments of Health and Human Services) under license 20–143 ex 08/09. For more detail see Wruck et al., 2015 (Table 1).

2.2. Cell culture

Primary fibroblast cells were cultured in high-glucose Dulbecco's Modified Eagle's Medium (DMEM) substituted with 1% Penicillin-Streptomycin (Penstrep), 1% Glutamine and 10% fetal calf serum (FCS) at 37 °C and 5% CO_2. IPS cells were cultured in Essential 8 (E8) Medium

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Table 1
Clinical background of donor primary fibroblast cell line.

	H0008
Gender	M
Age	61
BMI	46
Steatosis	40%
Operation	Bariatric surgery
Steatosis grade group	Obese, high steatosis
Waist	144
Hip	150
RR syst/blood pressure	154
T2DM	Y
Hypertension	Y
ALT	36
AST	26
GGT	31
Fasting glucose	95
Insulin	14.3
HOMA-IR	3.35
CHOL	159
TRIG	227
HDL	66

with 1% Penstrep on Matrigel or mouse embryonic fibroblasts (MEFs) as a feeder layer at 37 °C and 5% CO_2. All cell lines were split accordingly to their confluence once per week.

2.3. Derivation of iPSCs

The dermal fibroblast cell line S08 was reprogrammed into iPSCs by transduction of retroviruses encoding for OCT4, SOX2, KLF-4 and c-MYC as described in Juzefczuk J 2012.

2.4. DNA fingerprinting analysis

The STR analysis was performed by PCR amplification with the following primer sequences (5′–3): D17S1290 forward GCAACAGAGCAA GACTGTC, reverse GGAAACAGTTAAATGGCCAA; and D21S2055 forward AACAGAACCAATAGGCTATCTATC and reverse TACAGTAAATCACTTGGT AGGAGA.

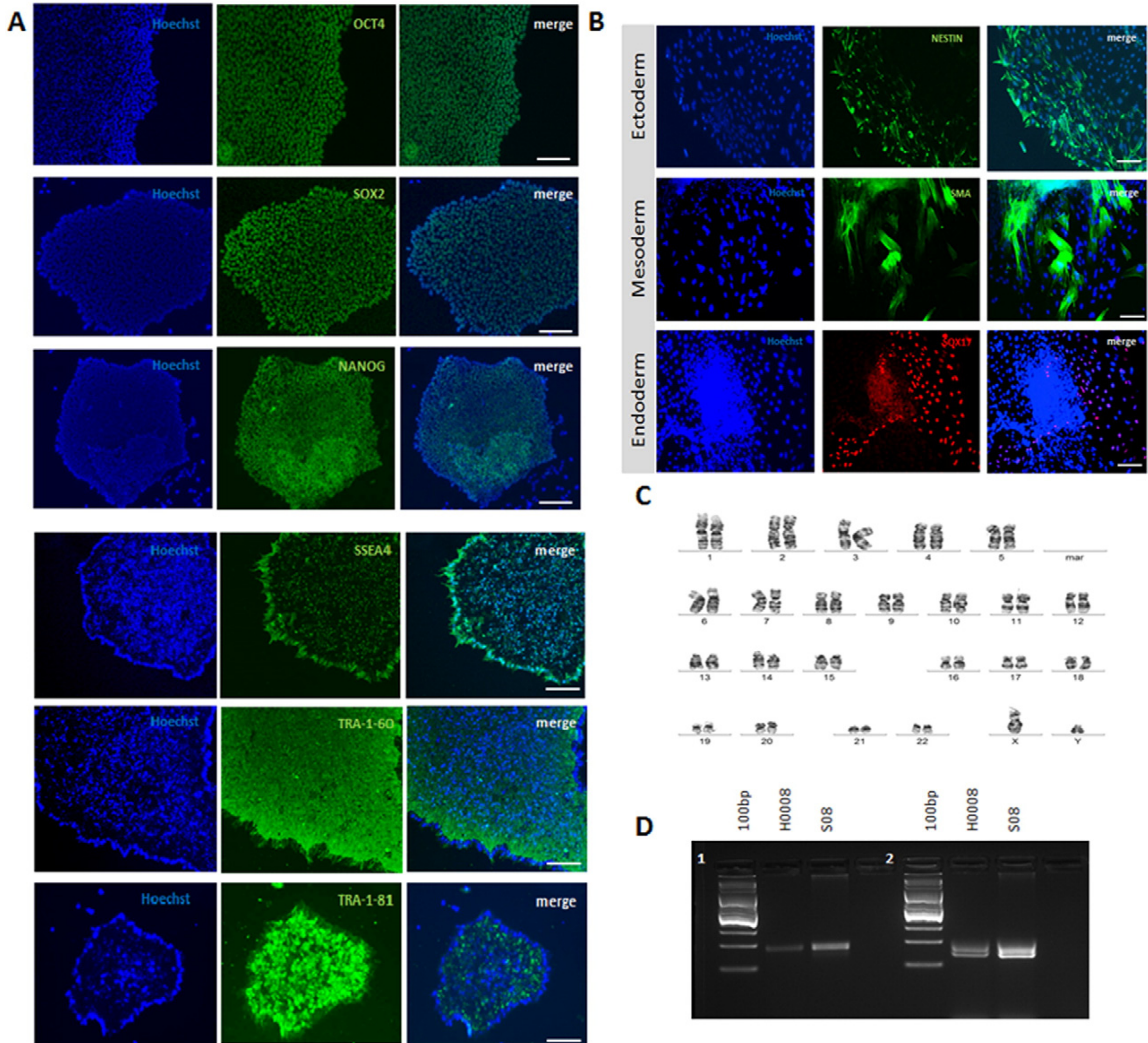

Fig. 1. Characterization of H0008-derived iPSC line S08. Characterization of the iPSC line S08 based on ICC for pluripotency transcription factorsOCT4, SOX2 and NANOG (A) and the cell surface markers SSEA4, TRA-1-60 and TRA-1-81 (A). (scale bar: 200 μm) and further verification of pluripotency by EB formation (B) and subsequent ICC for ectodermal (NESTIN), mesodermal (SMA) and endodermal (SOX17) markers. (scale bar: 100 μm). The cell type origin of the iPSC line S08 was performed by karyotype (C) and DNA-fingerprinting (D). DNA fingerprinting confirmed the origin of the iPSC line based on Primers D7S796 (1) and D21S2055 (2), while karyogram detected a 46,XY karyotype.

Fig. 2. Microarray-based transcriptome analysis. Microarray-based transcriptome analysis dendrogram of patient's fibroblasts (Fibroblasts), embryonic stem cells (H1_ES) and the retroviral-derived iPSC line (S08_iPSC).·The correlation co-efficient of H1_ES and S08_iPSC is XXXX.

2.5. Embryoid body formation

To confirm the pluripotent character of the cultured iPSCs EBs were generated as described in Matz and Adjaye (2015). To visualize differentiation immunocytochemistry-based detection of markers for all three germ layers was performed. As primary antibodies- SMA (1:100, Cell Signalling Technology®) was used to illustrate mesodermal differentiation. SOX17 (1:50, R&D Systems) for endodermal differentiation and Nestin (1:500, Sigma-Aldrich) for ectodermal differentiation. To visualize the localization of all cells nuclear Hoechst (1:5000) co-staining was performed Images were captured by using a fluorescence microscope (AxioVision).

2.6. Immunofluorescence-based detection of pluripotency-associated proteins

To confirm pluripotency immunocytochemistry (ICC) was performed. For the characterization of iPSCs primary antibodies for SOX2 (1:400, Cell Signalling Technology®), OCT4 (1:400, Cell Signalling Technology®), NANOG (1:800, Cell Signalling Technology®), TRA-1-60 (1:1000, Cell Signalling Technology®), TRA-1-81 (1:1000, Cell Signalling Technology®) and SSEA4, (1:1000, Cell Signalling Technology®) were used. After fixation of the cells with 4% paraformaldehyde (PFA) and blocking of unspecific binding-sites with a self-made blocking buffer (10% normal goat serum (NGS), 1% bovine serum albumin (BSA), 0.5% Triton (absent for surface marker), 0.05% Tween (absent for surface marker) in PBS) the primary antibodies were incubated over night at 4 °C. After several washing steps the secondary antibody incubation (1:500, anti-mouse-Cy3 (Thermo Fisher Scientific), anti-mouse-Alexa488 (Thermo Fisher Scientific), anti-rabbit-Alexa488 (Thermo Fisher Scientific)) could be performed for 1 h at room temperature (RT) without light exposure. To visualize the localization of all cells a nuclear Hoechst (1:5000) co-staining was accomplished. Images were captured by using a fluorescence microscope (AxioVision).

2.7. Karyotype analysis

Karyotype analysis was performed and evaluated at the Institute of Human Genetics and Anthropology, Heinrich-Heine-University, Düsseldorf.

2.8. Microarray-based transcriptome analysis

Global gene expression analysis was carried employing the Affymetrix microarray platform. For each sample, 1 μg quality-checked total RNA were used as input for the amplification and biotin labeling reactions, which precede bead chip hybridizations. RNA hybridizations was carried out by Biologisch-medizinisches Forschungszentrum (BMFZ), Heinrich-Heine University, Düsseldorf. The correlation values were calculated using the Gene Expression Module of the software R/Bioconductor (Affymetrix). Correlation coefficient of S08 iPSCs and H1 ESCs is 0.9287.

Acknowledgements

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