Stem Cell Research 17 (2016) 547–549

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Stem Cell Line

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

Marie-Ann Kawala [a], Martina Bohndorf [a], Nina Graffmann [a], Wasco Wruck [a], Kurt Zatloukal [b], James Adjaye [a,*]

[a] Institute for Stem Cell Research and Regenerative Medicine, Medical Faculty, Heinrich Heine University, 40225 Düsseldorf, Germany
[b] Institute of Pathology, Medical University of Graz, 8036 Graz, Austria

ARTICLE INFO

*Article history:*
Received 28 September 2016
Accepted 18 October 2016
Available online 20 October 2016

ABSTRACT

Primary fibroblasts from a low grade steatosis patient were reprogrammed by transduction of a combination of two episomal-based plasmids OCT4,SOX2, c-MYC and KLF4. iPSCs were characterized by immunocytochemistry, embryonic body-formation, DNA-fingerprint karyotype analysis and comparative transcriptome analyses with the human embryonic stem cell line H1 revealed a Pearsons correlation of 0.9251.



Resource table

| | |
|---|---|
| Name of stem cell construct | S12 |
| Institution | Institute for Stem Cell Research and Regenerative Medicine |
| Person who created resource | Martina Bohndorf |
| Contact person and email | James Adjaye, James.Adjaye@med.uni-duesseldorf.de |
| Date archived/stock date | January 2016 |
| 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 |

## Resource details

Primary dermal fibroblasts from H0012 (female, 50 years, low-steatosis) were reprogrammed by transduction of a combination of two episomal-based plasmids (7F1) expressing OCT4, SOX2, NANOG, LIN28, c-Myc and KLF-4 (H0012; Yu et al., 2011). iPSCs expressed the pluripotency-associated transcription factors OCT4, NANOG, SOX2 and cell surface markers SSEA-4, TRA-1-60, TRA-1-81. 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 both iPSC lines, while karyogram revealed a 46,XX,t(7;10)(q11;p12) karyotype for S12.

## Materials and methods

### *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 details see Wruck et al. (2015) (Table 1).

### *Cell culture*

For the cultivation of primary fibroblast cells 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% $CO_2$ was used. IPS cells were cultured in Essential 8 (E8) Medium 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 and replated weekly.

### *Derivation of iPSCs*

S12 was reprogrammed by the nucleofection of an episomal-based plasmid combination (7F1) expressing the reprogramming factors

* Corresponding author.
*E-mail address:* James.Adjaye@med.uni-duesseldorf.de (J. Adjaye).

http://dx.doi.org/10.1016/j.scr.2016.10.004

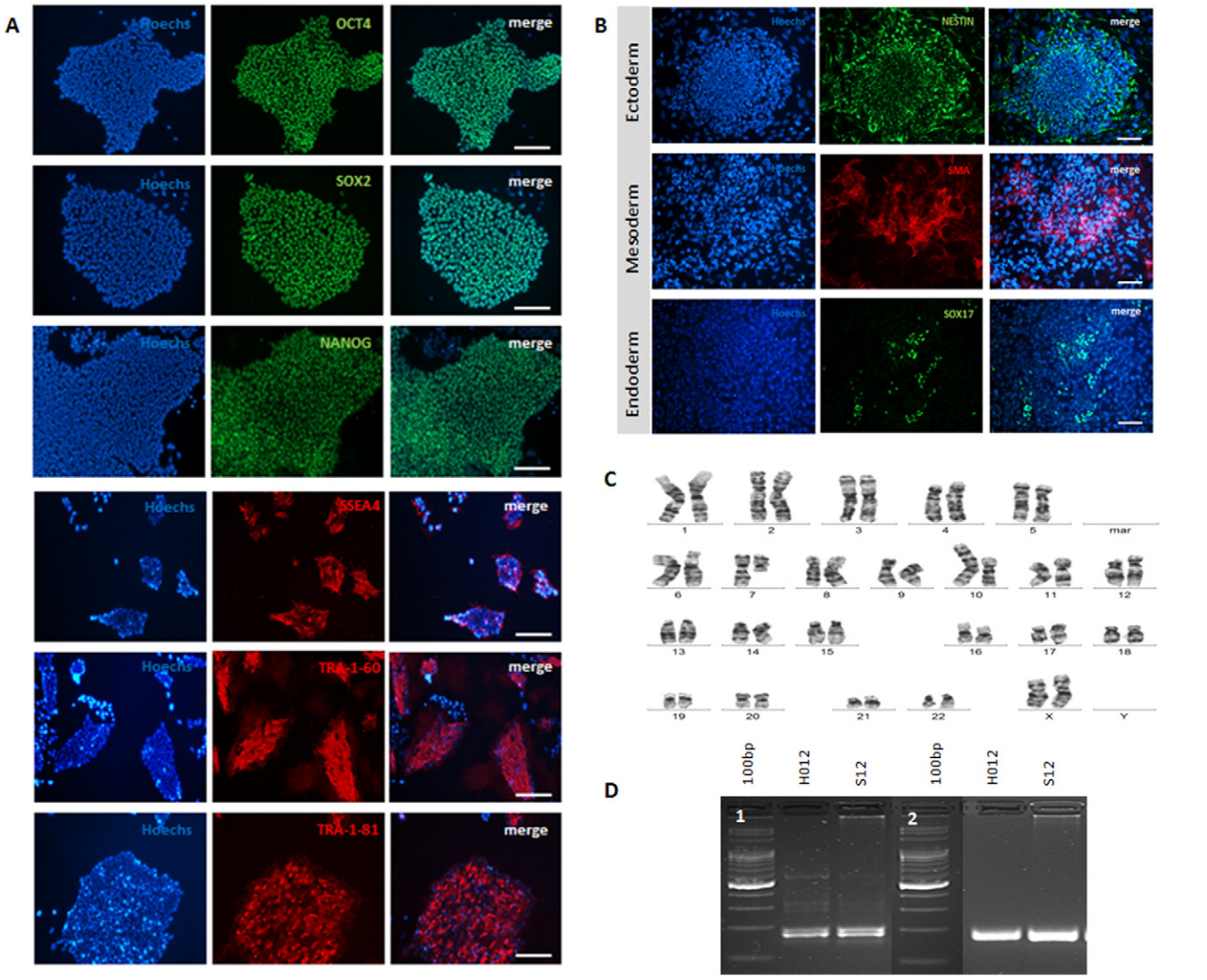


**Fig. 1.** Characterization of H0012-derived iPSC line S12. The iPSC line S12 was characterized by ICC for the pluripotency markers OCT4, SOX2 and NANOG (A) and SSEA4, TRA-1-60 and TRA-1-81 (A). (Scale bar: 200 μm). Pluripotency was confirmed by EB formation (B) and subsequent ICC for ectodermal (NESTIN), mesodermal (SMA) and endodermal (SOX17) markers. (Scale bar: 100 μm). The origin of iPSC line S12 was determined by karyotype (C) and DNA-fingerprinting (D). DNA fingerprinting confirmed the origin of the iPSC line employing Primers D7S796 (Jozefczuk et al., 2012) and D21S2055 (Yu et al., 2011), the karyogram revealed a 46,XX,t(7;10)(q11;p12) karyotype.

OCT4, SOX2, NANOG, KLF4, c-MYC and LIN28 (Yu et al., 2011). Nucleofection was achieved using a cell-specific Amaxa Cell Line Nucleofector Kit (Lonza) and transfected cells were supplemented with TGFβ-inhibitor (SB431245, Sigma, 2 μM), a MEK inhibitor (PD0325901, Sigma, 1 μM) and a GSK3β inhibitor (CHIR99021, Sigma, 3 μM).

*DNA fingerprinting analysis*

The STR analysis was performed by PCR amplification using the following primer sequences (5′–3′): D17S1290 forward GCAACAGAGCAAGACTGTC, reverse GGAAACAGTTAAATGGCCAA; and D21S2055 forward AACAGAACCAATAGGCTATCTATC and reverse TACAGTAAATCACTTGGTAGGAGA.

*Embryoid body formation*

To confirm pluripotency of the cultured iPSCs EB formation was performed as described in Matz and Adjaye (2015) and ICC to visualize differentiation we employed markers for all three germ layers. As primary antibodies SMA (1:100, Cell Signalling Technology®) was used to identify mesodermal differentiation. SOX17 (1:50, R&D Systems) for endodermal differentiation and Nestin (1:500, Sigma-Aldrich) ectoderm. Co-staining was accomplished using nuclear Hoechst (1:5000). Images were captured using a fluorescence microscope (AxioVision).

*Immunofluorescence-based detection of pluripotency associated proteins*

The following primary antibodies were used- 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®). 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)) was performed for 1 h at room temperature (RT) avoiding light exposure. To visualize the localization of all cells nuclear Hoechst (1:5000) co-staining was accomplished. Images were captured by using a fluorescence microscope (AxioVision).


**Fig. 2.** Microarray-based transcriptome analysis. Dendrogram generated from the transcriptomes of patient's fibroblasts (Fibroblasts), embryonic stem cells (H1_ES) and the retroviral-derived iPSC line (S12_iPS).

*Karyotype analysis*

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

*Microarray-based transcriptome analysis*

Transcriptome analysis was carried out on the Affymetrix microarray platform. For each sample, 1 μg quality-checked total RNA were used as input. RNA hybridizations were carried out by the 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 S12 iPSCs and H1 ESCs is 0.9251.

**Table 1**
Clinical background of donor primary fibroblast cell line.

| | H0012 |
|---|---|
| Gender | F |
| Age | 50 |
| BMI | 35 |
| Steatosis | 0% |
| Operation | Bariatric surgery |
| Steatosis grade group | Obese, low steatosis |
| Waist | 120 |
| Hip | 130 |
| RR syst/blood pressure | ND |
| T2DM | Y |
| Hypertension | Y |
| ALT | 17 |
| AST | 24 |
| GGT | 20 |
| Fasting glucose | 70 |
| Insulin | ND |
| HOMA-IR | ND |
| CHOL | 249 |
| TRIG | 57 |
| HDL | ND |

## Acknowledgements

J.A. acknowledges support from the medical faculty of Heinrich–Heine Universität Düsseldorf.

## References

Jozefczuk, J., Kashofer, K., Ummanni, R., Henjes, F., Rehman, S., Geenen, S., Wruck, W., Regenbrecht, C., Daskalaki, A., Wierling, C., Turano, P., Bertini, I., Korf, U., Zatloukal, K., HV, W., Lehrach, H., Adjaye, J., 2012. A systems biology approach to deciphering the etiology of steatosis employing patient-derived dermal fibroblasts and iPS cells. Front. Physiol. 2012 (3), 339.

Matz, P., Adjaye, J., 2015. Generation of iPSC line epiHUVEC from human umbilical vein endothelial cells. Stem Cell Res. 15, 581–583.

Wruck, W., Kashofer, K., Rehman, S., Daskalaki, A., Berg, D., Gralk, E., Jozefczuk, J., Drews, K., Pandey, V., Regenbrecht, C., Wierling, C., Turano, P., Korf, U., Zatloukal, K., Lehrach, H., HV, W., Adjaye, J., 2015. Multi-omic profiles of human non-alcoholic fatty liver disease tissue highlight heterogenic phenotypes. Sci Data. 2:150068. http://dx.doi.org/10.1038/sdata.2015.68.

Yu, J., Chau, K.F., Vodyanik, M.A., Jiang, J., Jiang, Y., 2011. Efficient feeder-free episomal reprogramming with small molecules. PLoS One 6, e17557.