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Lab resource: Stem Cell Line

Generation of a human induced pluripotent stem cell (iPSC) line from a patient carrying a P33T mutation in the *PDX1* gene

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ABSTRACT

Homozygous loss-of-function mutations in the gene coding for the homeobox transcription factor *PDX1* leads to pancreatic agenesis, whereas certain heterozygous point mutations are associated with Maturity-Onset Diabetes of the Young 4 (MODY4) and Type 2 Diabetes Mellitus (T2DM). To understand the pathomechanism of MODY4 and T2DM, we have generated iPSCs from a woman with a P33T heterozygous mutation in the transactivation domain of PDX1. The resulting *PDX1* P33T iPSCs generated by episomal reprogramming are integration-free, have a normal karyotype and are pluripotent *in vitro* and *in vivo*. Taken together, this iPSC line will be useful to study diabetes pathomechanisms.

1. Resource table

Name of stem cell line	*PDX1* P33T iPSC1
Institution	Institute of Diabetes and Regeneration Research
Person who created resource	Xianming Wang
Contact person and email	Heiko Lickert heiko.lickert@helmholtz-muenchen.de
Date archived/stock date	April 2014
Origin	Human dermal fibroblasts
Type of resource	Induced pluripotent stem cells from a woman carrying a *PDX1* P33T mutation
Sub-type	Cell line
Key transcription factors	OCT4, SOX2, NANOG, LIN28, KLF4, and L-MYC
Authentication	Identity and purity of cell line confirmed
Link to related literature	Not available
Information in public databases	Not available
Ethics	Informed written consent obtained

2. Resource details

The Tübingen Family Study for T2DM (TÜF study, N = 2500) was screened for known rare mutations in the MODY4 gene *PDX1*. By mass spectrometry-based genotyping, one heterozygous *PDX1* P33T carrier could be identified and recruited for full-thickness skin biopsy. From the biopsy material, the epidermal layer was separated from the dermis by dispase digestion and fibroblasts were isolated from the dermis by trypsin digestion using an established protocol (see Materials and methods section). The *PDX1* P33T fibroblasts were expanded using commercially available growth media and deep-frozen in liquid nitrogen. From this collection of dermal fibroblasts, samples were tested for the presence of viruses pathogenic to humans (HBV, HCV, HIV) as well as for the presence of mycoplasma. All cultures were found to be negative for all of these potential contaminants. *PDX1* P33T primary fibroblasts were reprogrammed into iPSCs using nucleofection with three episomal plasmids encoding human *OCT4*, *SOX2*, *NANOG*, *LIN28*, *KLF4*, and *L-MYC* (Fig. 1B). The reprogramming protocol (transfection, reprogramming and expansion) is shown in Fig. 1C. Three weeks after transfection, many colonies with human embryonic stem cell (hESC)-

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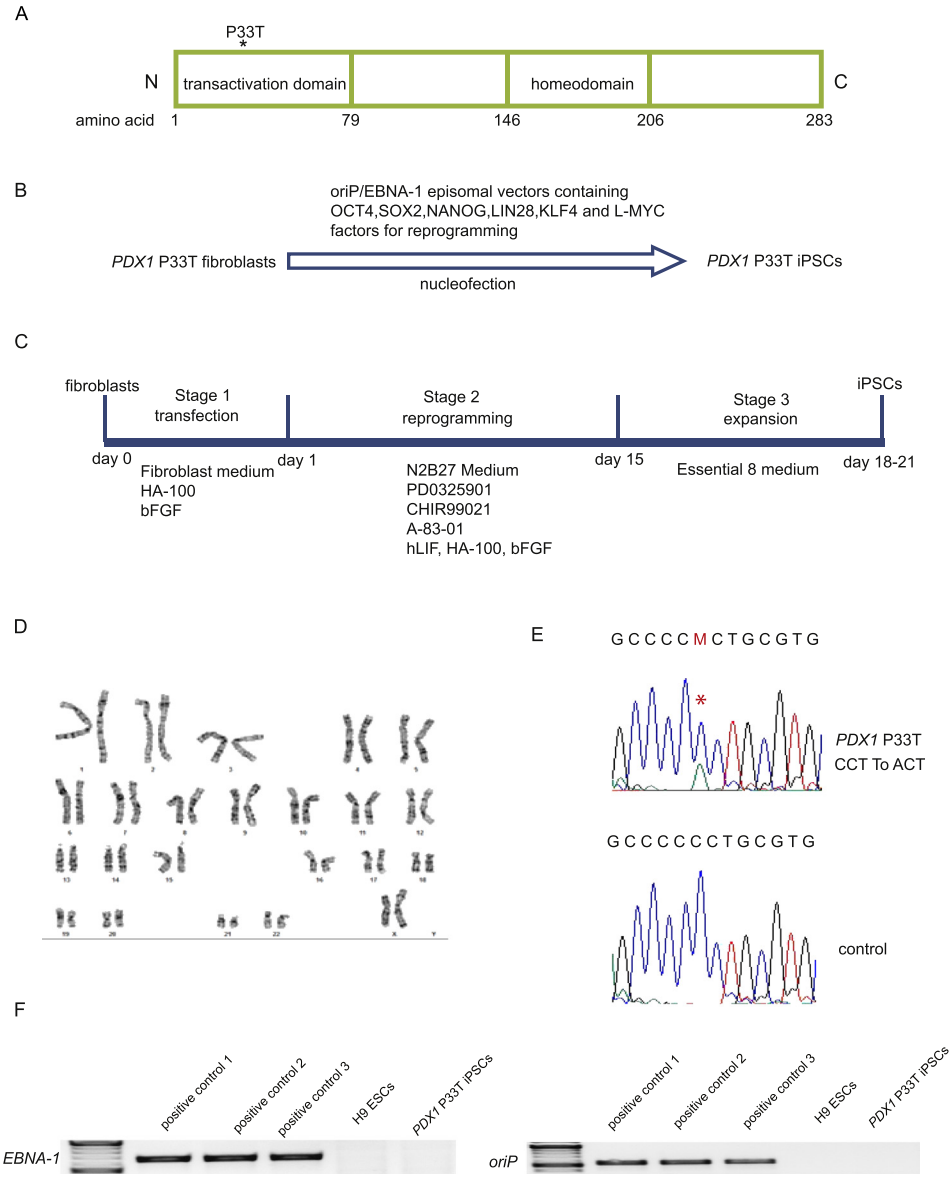

Fig. 1. Generation of *PDX1* P33T iPSCs. (A) An overview of the PDX1 protein structure. (B) Scheme shows the reprogramming factors for the generation of the *PDX1* P33T iPSC line. (C) Reprogramming protocol of skin fibroblasts into integration-free iPSCs using episomal vectors. (D) Normal karyotype (46, XX) of one *PDX1* P33T iPSC clone. (E) Sequencing result shows heterozygous C>A mutation in *PDX1*. (F) Semi-quantitative PCR confirms that episomal vectors did not integrate into the genomic DNA of the selected *PDX1* P33T iPSC clone. H9 ESCs were used as a negative control, whereas transfected fibroblasts at day 6 were used as positive controls.

like morphology appeared that were hand picked by day 21, expanded and frozen down as individual iPSC lines. The analysis of one of these iPSC lines confirmed that *PDX1* P33T fibroblasts were successfully reprogrammed into iPSCs with a normal karyotype (Fig. 1D) and containing the C>A mutation causing a P33T amino acid exchange in the transactivation domain (Fig. 1A, E). Integration of the exogenous transgenes in the genome of iPSCs was excluded by semi-quantitative PCR (Fig. 1F). To confirm that this iPSC line was pluripotent, we performed immunofluorescence staining for the pluripotency transcription factors OCT4 and SOX2, as well as for the hESC surface markers SSEA-3, SSEA-4 and TRA-1-81, which were all expressed in the *PDX1* P33T iPSC line (Fig. 2A). To further demonstrate pluripotency of the selected iPSC line we injected 2×10^6 cells subcutaneously into NOD/SCID mice to generate teratomas. The histological analysis of the induced teratomas revealed that all tissues from all three germ layers were differentiated confirming pluripotency in this *in vivo* assay (Fig. 2B). Taken together, we have successfully reprogrammed *PDX1* P33T dermal fibroblasts into pluripotent iPSCs that can be used to study a point mutation in the transactivation domain of PDX1 to study the development of diabetes.

3. Materials and methods

3.1. Ethics statement

The study adhered to the Declaration of Helsinki. All participants gave informed written consent, and the study protocols were approved by the local ethics board (Ethics Committee of the Eberhard Karls University Tübingen).

3.2. Study participants

The ongoing TÜF study currently includes >3000 non-related individuals at risk for type-2 diabetes mellitus (T2DM), *i.e.*, healthy subjects with