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Lab resource

Generation of spinocerebellar ataxia type 3 patient-derived induced pluripotent stem cell line SCA3.B11

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ABSTRACT

Spinocerebellar ataxia type 3 (SCA3) is a dominantly inherited neurodegenerative disease caused by an expansion of the CAG-repeat in *ATXN3*. In this study, induced pluripotent stem cells (iPSCs) were generated from SCA3 patient dermal fibroblasts by electroporation with episomal plasmids encoding *L-MYC*, *LIN28*, *SOX2*, *KLF4*, *OCT4* and short hairpin RNA targeting *P53*. The resulting iPSCs had normal karyotype, were free of integrated episomal plasmids, expressed pluripotency markers, could differentiate into the three germ layers *in vitro* and retained the disease-causing *ATXN3* mutation. Potentially, this iPSC line could be a useful tool for the investigation of SCA3 disease mechanisms.

Resource table:	
Name of Stem Cell line	SCA3.B11
Institution	University of Copenhagen
Person who created resource	Susanne K. Hansen
Contact person and email	Jørgen E. Nielsen, jnielsen@sund.ku.dk
Date archived/stock date	Feb 5, 2013
Origin	Human skin fibroblasts
Type of resource	Induced pluripotent stem cells derived from a patient with spinocerebellar ataxia type 3 (SCA3), CAG-repeat lengths: 14/74
Sub-type	Cell line
Key transcription factors	Episomal plasmids containing *SOX2*, *L-MYC*, *KLF4*, *LIN28*, *OCT4* and *shP53* (Okita et al. 2011)
Authentication	Identity and purity of the cell line were confirmed by karyotyping, integration analysis, pluripotency analysis and confirmation of the CAG-repeat expanding mutation in *ATXN3* (Fig. 1)
Link to related literature	http://www.nature.com/nature/journal/v480/n7378/full/nature10671.html (Koch et al. 2011)
Information in public databases	Not available
Ethics	The study was approved by the regional scientific ethical committee in the Capital Region of Denmark and informed consent was obtained from the patient (H-4-2011-157).

1. Resource details

Spinocerebellar ataxia type 3 (SCA3) is a dominantly inherited neurodegenerative disease caused by a CAG-repeat expanding mutation of the gene *ATXN3* encoding ataxin-3. CAG-repeat length of mutated alleles can vary from 45 to 87 repeats (Matos et al. 2011). In the current study, dermal fibroblasts (H249) were derived from a skin biopsy of a 58-year-old man with spinocerebellar ataxia type 3 (SCA3) with 78 CAG-repeats in the disease allele of *ATXN3*.[1] Patient fibroblasts were reprogrammed to iPSCs by electroporation with three episomal plasmids encoding human *L-MYC* and *LIN28*, *SOX2* and *KLF4*, and *OCT4* combined with a short hairpin RNA for *P53* (*shP53*). The iPSC line described in this publication was termed SCA3.B11. An additional iPSC line termed SCA3.B1 was derived from the same patient and characterized (data not shown). SCA3.B11 had a numerically and structurally normal karyotype (46, XY) (Fig. 1A) and no integration of reprogramming plasmids (Fig. 1B). The pluripotency genes *OCT4*, *NANOG*, *SOX2* and *LIN28* were upregulated in iPSCs compared to patient fibroblasts (Fig. 1C) and the gene expression levels were comparable to those of a previously described positive control iPSC line (Rasmussen et al., 2014). In

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[1] This CAG-repeat length was determined in blood cells at the time of diagnose and differs from iPSCs and fibroblasts probably because of somatic mosaicism.

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agreement, all SCA3.B11 iPSCs expressed the pluripotency proteins OCT4, NANOG, TRA-1-60, TRA-1-81, SSEA-3 and SSEA-4 (Fig. 1D), illustrating the purity of the iPSC line. Pluripotency was supported by the ability of SCA3.B11 to differentiate to cells from the three germ layers *in vitro*, as shown by fluorescent immunocytochemistry displaying expression of the endodermal marker α-fetoprotein (AFP), the mesodermal marker smooth muscle actin (SMA) and the ectodermal marker β-III-tubulin (βIIItub) (Fig. 1E). The identity of SCA3.B11 was confirmed by demonstrating expression of both normal and expanded ataxin-3 proteins by western blot (Fig. 1F). Furthermore, the lengths of the CAG-repeats in the two ataxin-3 alleles were determined to 14 and 74 repeats in both patient iPSCs and fibroblasts by fragment length analysis (data not shown).

2. Materials and methods

2.1. Reprogramming of fibroblasts to iPSCs

Written informed consent was obtained from the SCA3 patient and the study was approved by the regional scientific ethical committee in the Capital Region of Denmark (H-4-2011-157). A skin biopsy was obtained from the forearm of a 58-year-old male SCA3 patient, dissected

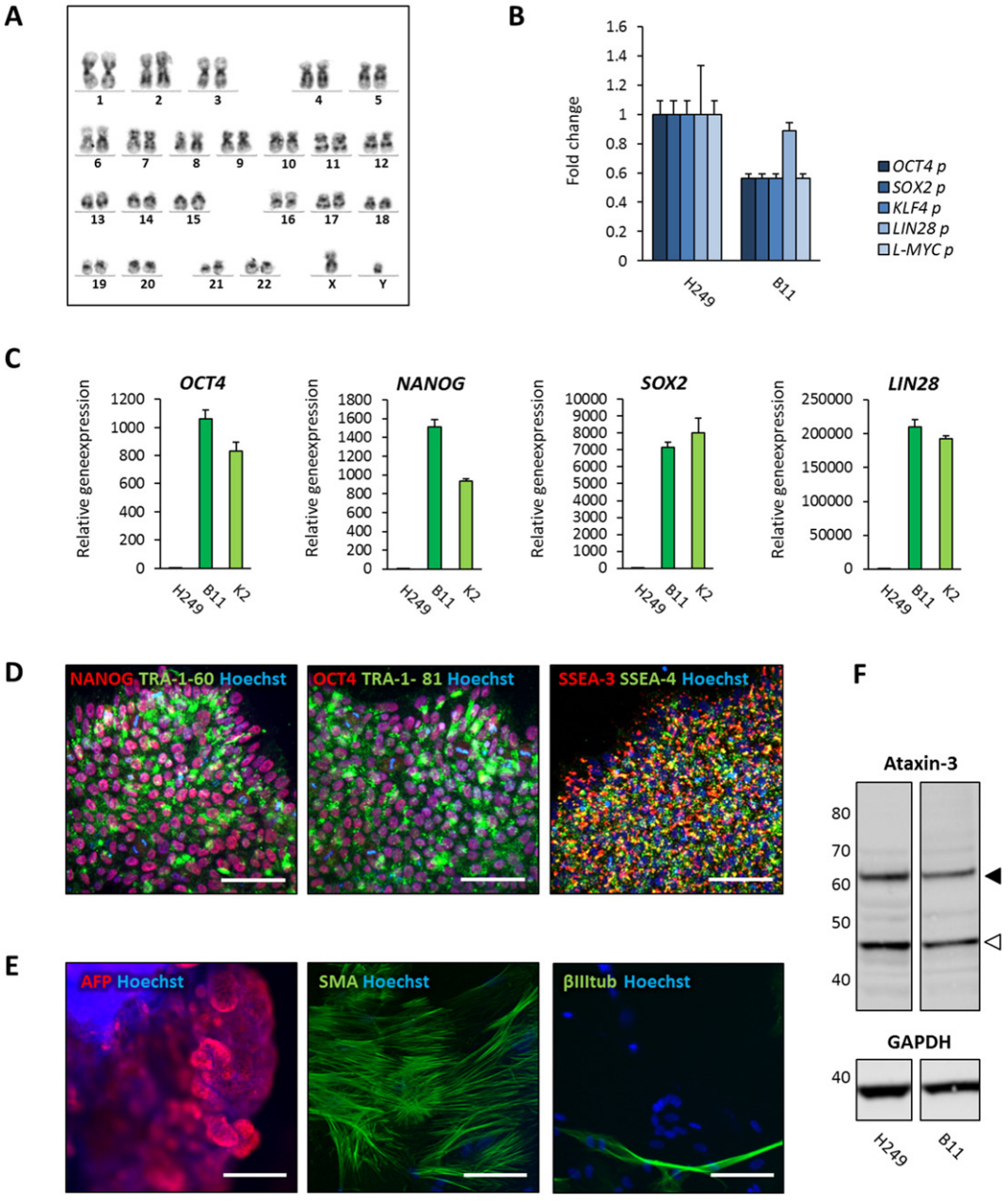

Fig. 1. A. Karyotyping. Representative metaphase of SCA3.B11 iPSCs. **B.** Integration analysis. Patient fibroblasts (H249) and SCA3.B11 (B11) iPSCs were harvested in duplicates and DNA was isolated for qRT-PCR with reprogramming plasmid specific primers. C_T-values were normalized to the geometric mean of *Hsp90AB1*, *GUSB* and *RPL13A* and fold change was calculated relative to fibroblasts using the $\Delta\Delta C_T$ –method (Mean ± S.D). **C.** Gene expression of pluripotency markers. Patient fibroblasts, SCA3.B11 and K2_shP53 (K2) iPSCs (Rasmussen et al., 2014) were harvested in triplicates and reverse transcribed to cDNA for qRT-PCR. C_T-values were normalized to the geometric mean of *Hsp90AB1*, *GUSB* and *RPL13A* and gene expression was calculated relative to fibroblasts using the $\Delta\Delta C_T$ -method (Mean ± S.D). **D.** Protein expression of pluripotency markers. Pluripotency markers were stained by fluorescent immunocytochemistry on SCA3.B11 iPSCs. Cell nuclei were stained with Hoechst. Scalebars: 100 μm. **E.** *In vitro* differentiation. Fluorescent immunocytochemistry of the endodermal marker α-fetoprotein (AFP), the mesodermal marker smooth muscle actin (SMA) and the ectodermal marker β-III-tubulin (βIIItub) in plated iPSC-derived embryoid bodies. Scalebars: 100 μm. **F.** Expression of ataxin-3 disease mutation. Western blot showing the protein expression of ataxin-3 in patient fibroblasts and SCA3.B11. White arrowheads indicate wild type ataxin-3, while black arrowheads show the expanded form of ataxin-3. GAPDH was used to ensure equal loading.