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Lab Resource: Stem Cell Line

Generation of spinocerebellar ataxia type 3 patient-derived induced pluripotent stem cell line SCA3.A11

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ABSTRACT

Spinocerebellar ataxia type 3 (SCA3) is a dominantly inherited neurodegenerative disease caused by a CAG-repeat expanding mutation in *ATXN3*. We generated induced pluripotent stem cells (iPSCs) from a SCA3 patient by electroporation of dermal fibroblasts 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 genomically integrated episomal plasmids, expressed pluripotency markers, could differentiate into the three germ layers *in vitro* and retained the disease-causing *ATXN3* mutation. This iPSC line could be useful for the investigation of SCA3 disease mechanisms.

Resource table.

Name of Stem Cell line	SCA3.A11
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: 23/83
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).

Resource details

Spinocerebellar ataxia type 3 (SCA3) is a dominantly inherited neurodegenerative disease caused by a mutation expanding the CAG-repeat of the *ATXN3* gene encoding ataxin-3. CAG-repeat length of disease alleles can vary from 45 to 87 repeats (Matos et al., 2011). In this study, dermal fibroblasts (named H241) were obtained from a 17-year-old man with spinocerebellar ataxia type 3 (SCA3) with 83 CAG-repeats in the mutant *ATXN3* allele. The fibroblasts were reprogrammed into 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 named SCA3.A11. An additional clone from the same patient termed SCA3.A8 was isolated and characterized (data not shown). SCA3.A11 had a structurally and numerically normal karyotype (46, XY) (Fig. 1A) and no integration of episomal reprogramming plasmids (Fig. 1B). The expression of the pluripotency genes *OCT4*, *NANOG*, *SOX2* and *LIN28* was upregulated in iPSCs compared to patient fibroblasts (Fig. 1C) and the gene expression levels were comparable to those of a characterized positive control iPSC line (Rasmussen et al., 2014). Accordingly, all SCA3.A11 iPSCs stained positive for the pluripotency markers 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 capability of SCA3.A11 to differentiate into the three germ layers

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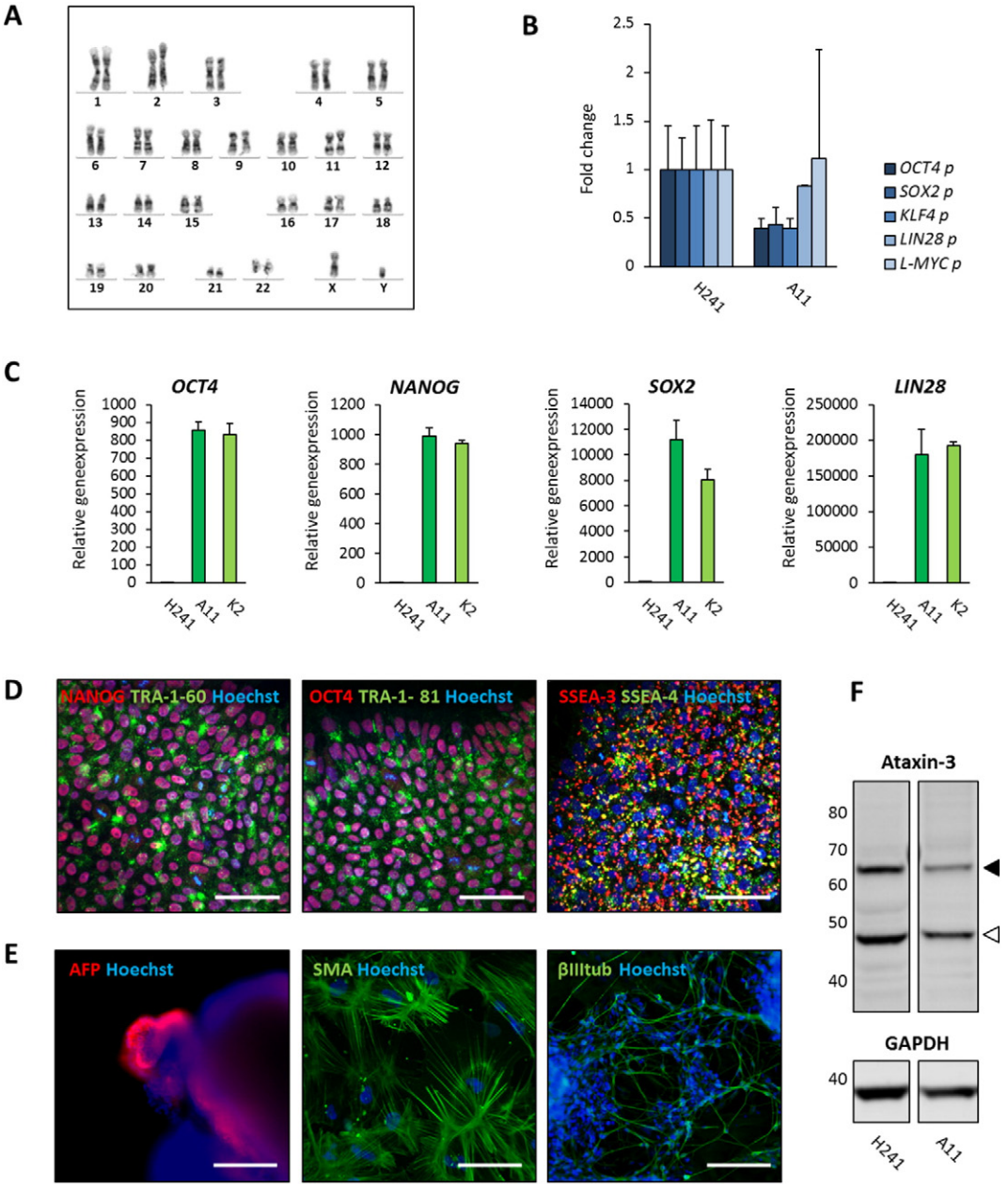

Fig. 1. A. Karyotyping. Representative karyotype of SCA3.A11 iPSCs. B. Integration analysis. Patient fibroblasts (H241) and SCA3.A11 (A11) iPSCs were harvested in duplicates and DNA was isolated for qRT-PCR with reprogramming plasmid specific primers. C_T-values were normalized to the geometric mean 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.A11 iPSCs and a previously characterized iPSCs line K2_shP53 (K2) (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 expression was calculated relative to fibroblasts using the $\Delta\Delta C_T$-method (Mean ± S.D). D. Protein expression of pluripotency markers. Fluorescent immunocytochemistry for pluripotency markers was performed on SCA3.A11 iPSCs. Cell nuclei were stained with Hoechst. Scalebars: 100 μm. E. *In vitro* differentiation. Fluorescent immunocytochemistry showing expression 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.A11. White arrowheads show wild type ataxin-3, while black arrowheads indicate the expanded form of ataxin-3. GAPDH was used as a loading control.

in vitro, as confirmed by fluorescent immunocytochemistry showing 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.A11 was confirmed by verifying expression of both normal and expanded ataxin-3 proteins by western blot (Fig. 1F). Additionally, the CAG-repeat lengths of the two ataxin-3 alleles were determined to be 23 and 83 repeats in patient fibroblasts and iPSCs by fragment length analysis (data not shown).

Materials and methods

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 taken from the forearm of a 17-year-old male SCA3 patient, dissected and left in fibroblast medium consisting of Dulbecco's modified eagle's