Stem Cell Research 30 (2018) 61–68

Contents lists available at ScienceDirect

Stem Cell Research

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

Motor neuron differentiation of iPSCs obtained from peripheral blood of a mutant *TARDBP* ALS patient

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ARTICLE INFO

Keywords:
Amyotrophic Lateral Sclerosis (ALS)
Induced Pluripotent Stem Cells (iPSCs)
Peripheral blood
TDP43
Motor neuron

ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disease, mainly affecting the motor neurons (MNs) and without effective therapy. Drug screening is hampered by the lack of satisfactory experimental and pre-clinical models. Induced pluripotent stem cells (iPSCs) could help to define disease mechanisms and therapeutic strategies as they could be differentiated into MNs, otherwise inaccessible from living humans. In this study, given the seminal role of TDP-43 in ALS pathophysiology, MNs were obtained from peripheral blood mononuclear cells-derived iPSCs of an ALS patient carrying a p.A382T *TARDBP* mutation and a healthy donor. Venous samples were preferred to fibroblasts for their ease of collection and no requirement for time consuming extended cultures before experimentation. iPSCs were characterized for expression of specific markers, spontaneously differentiated into primary germ layers and, finally, into MNs. No differences were observed between the mutated ALS patient and the control MNs with most of the cells displaying a nuclear localization of the TDP-43 protein. In conclusion, we here demonstrated for the first time that human *TARDBP* mutated MNs can be successfully obtained exploiting the reprogramming and differentiation ability of peripheral blood cells, an easily accessible source from any patient.

1. Introduction

An important limitation in the field of neurodegenerative disorders is the difficulty to translate information provided by preclinical research into effective new treatments for patients. This unmet need is mainly due to the scarcity of adequate experimental models. This is particularly true for amyotrophic lateral sclerosis (ALS), where motor neurons (MNs) and other nervous tissues affected by disease processes are difficult to obtain from alive patients. In addition, when post mortem brain samples are available, differentiated neurons do not anyway undergo cell division, resulting in a limited utility for *in vitro* functional studies.

The majority of ALS patients present a sporadic form of the disease, while around 10% are familial cases with > 20 causative genes identified so far among which *SOD1*, *C9ORF72, TARDBP* and *FUS* represent the main involved (Chen et al., 2013). Several transgenic animal models have been developed in order to investigate the different pathomechanisms of ALS and to test future possible therapies: the first and most commonly used was the human transgenic *SOD1* mouse (Gurney et al., 1994). Later, hemizygous and homozygous mice expressing wild-type and mutated human TDP-43 (Stallings et al., 2010), (Xu et al., 2010) together with mice carrying the *C9ORF72* GGGGCC repeat expansion (Chew et al., 2015) were generated. Albeit animal models have been useful for investigation of some ALS pathological mechanisms, they recapitulate only partial aspects of the disease mostly reproducing familial forms of ALS. Indeed, drug effects in SOD1 transgenic mice were rarely able to predict the same efficacy in humans (Ludolph et al., 2007), (Ludolph et al., 2010). This discrepancy may be explained by the diversity in both structure and development of rodent and human brains together with the absence of naturally occurring ALS in mice. Consequently, animal models may not be the most adequate tool to fully represent the various phenotypes of human ALS. Cellular model systems, such as NSC-34 (Cashman et al., 1992), SH-SY5Y (Biedler et al., 1973) or HEK293T (Graham et al., 1977) have also been widely used and useful to study ALS pathomechanisms *in vitro*. However, they are mostly tumor-derived or engineered cells, not completely

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https://doi.org/10.1016/j.scr.2018.05.009
Received 30 January 2018; Received in revised form 24 April 2018; Accepted 16 May 2018
Available online 17 May 2018

mimicking the properties of primary neuronal cells. Recent innovative *in vitro* model, such as induced pluripotent stem cells (iPSCs) (Takahashi and Yamanaka, 2006), has represented a turning point in the development of experimental disease models and can help to improve current knowledge of the pathogenic mechanisms of ALS after MN differentiation. In ALS, iPSCs have been generated from both mutated and sporadic patients, extensively expanded in culture and differentiated into cell types such as MNs and astrocytes (Jaiswal, 2017). As the main hallmark of ALS is the progressive degeneration of spinal cord MNs, their generation is essential for *in vitro* studies. MNs derived from iPSCs have been shown to exhibit equivalent functional characteristics of *in vivo* MNs: they express specific markers by immunostaining, are able to project axons and are responsive to glutamate agonists. They therefore represent an ideal tool for modeling MN degenerative diseases (Amoroso et al., 2013). The most widely used somatic cells for iPSCs generation are fibroblasts (Gonzalez et al., 2011) but several other cell types have been exploited, for example peripheral blood cells (Huang, 2010). These cells present many advantages compared to fibroblasts as they do not need to be cultured *in vitro* for several passages, avoiding the risk of genomic changes accumulation. In addition, peripheral blood collection is less invasive than skin biopsy.

The multifunctional RNA-binding protein TDP-43 is involved in RNA metabolism including splicing, transcription and mRNA transport (Ratti and Buratti, 2016). Its localization is primarily nuclear, however the protein shuttles between nucleus and cytoplasm. In both familial and sporadic ALS, as well as in frontotemporal dementia, pathological TDP-43-positive cytoplasmic inclusions are observed in affected brains (Arai et al., 2006), (Neumann et al., 2006). Moreover, mutations in the TDP-43-encoding gene *TARDBP* account for about 5% of familial and 1% of sporadic ALS cases and the missense p.A382T variant is the most frequently occurring one in the Italian population (Corrado et al., 2009). In this study we report, for the first time, the reprogramming of peripheral blood cells from an ALS patient carrying the *TARDBP* p.A382T mutation into iPSCs. These iPSCs were fully characterized for their pluripotency and successfully differentiated into MNs similarly to healthy control-derived iPSCs.

2. Materials and methods

2.1. Participants and samples

This study was approved by the ethics committee of IRCCS Istituto Auxologico Italiano. Written informed consent was obtained from the participants. All research was performed in accordance with relevant guidelines. We selected an ALS patient carrying the TARDBP p.A382T mutation (see details in Table 1) and an unrelated healthy donor as control. Both were females, 63 and 49 years old, respectively. PBMCs were isolated from peripheral blood samples by density gradient centrifugation on Ficoll-PaqueTM Plus (GE Healthcare, Chicago, IL, USA).

2.2. Generation of iPSCs

To generate iPSCs, 5×10^5 PBMCs were seeded in a 24-well plate in StemPro™-34 medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with SCF (100 ng/ml), FLT-3 (100 ng/ml), IL-3 (20 ng/ml) and IL-6 (20 ng/ml) cytokines (all from Peprotech, London, UK). Half medium was daily changed and fresh cytokines added to the cell suspension. After four days, transduction was performed using CytoTune-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific), by adding the Klf4, Oct4, Sox2 and c-Myc virus, following manufacturer's instructions (KOS (Klf4–Oct3/4–Sox2) MOI = 5, hcMyc MOI = 5 and hKlf4 MOI = 3). Three days later, transduced cells were transferred on Mouse Embryonic Fibroblasts (MEF) feeder layer, and grown in StemPro™-34 medium without the cytokines for four days. Thereafter cultures were carried on in Essential 8 medium (Thermo Fisher Scientific). Spent medium was daily replaced with fresh medium and the culture vessels monitored for the emergence of iPSC colonies. Twenty days post-transduction, colonies ready for transfer were individually picked and seeded onto Geltrex (Thermo Fisher Scientific)-coated dishes. Colonies, passaged using an EDTA 0.5 μM solution, were expanded in Essential 8 medium for at least six passages before being characterized and differentiated.

Table 1
Characteristics of the ALS TDP-43 p.A382T patient.

Clinical parameters	Patient data
Dementia	No (normal ECAS score)
ALS-FRS-R score	40/48 at diagnosis, 31/48 at blood sampling
Disease duration	> 5 years
I MN involvement	Yes
II MN involvement	Yes
Onset symptoms	Weakness in drawing head backwards (weakness of the rectus capitis posterior muscle)

2.3. Genotyping of patient-derived iPSCs

Genomic DNA was extracted from mutant iPSC using DNAzol Reagent (Invitrogen). TARDBP exon 6 was amplified by PCR using the following primers: FOR_tgcttgtaatctaagttttgttg and REV_aaatttgaattcccaccattc. The amplicon was sequenced with BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI 3500 Genetic Analyzer (Applied Biosystems) and compared with the reference sequence NM_007375.3 GRCh37/hg19.

2.4. In vitro spontaneous differentiation of iPSCs

To test the *in vitro* spontaneous differentiation ability of the established iPSCs, embryoid bodies (EBs) formation was performed by gently resuspending iPSCs colonies in non-tissue culture-treated plates in HuES medium (DMEM/F12, 20% knock-out serum replacement, 2 mM L-glutamine, 10 U/ml penicillin, 10 μg/ml streptomycin, 0.1 mM MEM NEAA, 110 μM β-mercaptoethanol) (all from Thermo Fisher Scientific). Medium was changed daily and after 7 days, EBs were collected and plated on Geltrex-coated plates in Essential 8 medium. Expression of the three germ layers specific markers was evaluated by immunofluorescence (βIII tubulin for ectoderm, desmin for mesoderm and alpha-fetoprotein (AFP) for endoderm).

2.5. Motor neuron differentiation

A modified protocol from Amoroso et al. (Amoroso et al., 2013) was used for MN differentiation. A schematic representation of the protocol is shown in Fig. 2a. Briefly, iPSCs were grown in a 100 mm dish until confluence, harvested and placed into non-cell culture-treated dishes. To obtain EBs, for the first 2 days, cells were allowed to grow in suspension in HuES medium supplemented with 20 ng/ml basic fibroblast growth factor (FGF) (Peprotech) and 20 μM Rho-associated kinase (ROCK) inhibitor Y27632 (Selleckchem, Houston, TX, USA) in order to enhance single cell survival. The third day, neuralization was induced by the addition of 10 μM SB431542 and 0.2 μM LDN193189 (both from Stemgent, Cambridge, NY, USA) to the cultures. The fourth day, EBs were switched to neural induction medium (DMEM/F12, 2 mM L-glutamine, 10 U/ml penicillin, 10 μg/ml streptomycin, 0.1 mM MEM NEAA, 2 μg/ml heparin (Sigma-Aldrich, Saint Louis, MO, USA), 1% N2 supplement (Thermo Fisher Scientific)), supplemented with 20 μM ROCK inhibitor, 0.4 μg/ml ascorbic acid (AA) (Sigma-Aldrich), 1 μM retinoic acid (RA) (Sigma-Aldrich), 10 ng/ml brain-derived neurotrophic factor (BDNF) (Peprotech). SB431542 and LDN193189 were added until day 7 when cultures were supplemented with 1 μM smoothened agonist (SAG) (Merck) and 0.5 μM purmorphamine (Pur) (Sigma-Aldrich). EBs were grown for additional ten days with a

medium change every alternate day. At day 17, EBs were dissociated with 0.05% trypsin (Sigma-Aldrich) and plated on poly-lysine (Sigma-Aldrich)/laminin (Thermo Fisher Scientific)-coated coverslips in 24-well plates at a concentration of 5×10^5 cells/well. Cells were cultured in neural differentiation medium (Neurobasal (Thermo Fisher Scientific), 2 mM L-glutamine, 10 U/ml penicillin, 10 µg/ml streptomycin, 0.1 mM MEM NEAA, 1% N2 supplement), supplemented with 2% B27 (Thermo Fisher Scientific), 1 µg/ml laminin (Lam), 25 µM glutamate (Glu) (Sigma-Aldrich), 0.4 µg/ml ascorbic acid, 10 ng/ml glial-derived neurotrophic factor (GDNF) and 10 ng/ml ciliary neurotrophic factor (CNTF) (both from Peprotech). MNs were allowed to differentiate for 10 days and finally fixed for immunofluorescence or recovered for RNA extraction.

2.6. Reverse transcription-PCR

Total RNA from both iPSCs and iPSCs-derived MNs was isolated using the TRIzol reagent (Thermo Fisher Scientific) following manufacturer's instructions and reverse transcribed into cDNA using Super Script II reverse transcriptase (Thermo Fisher Scientific). Briefly, a first incubation with ribonuclease inhibitor and DNAse was performed at 37 °C for 20 min to remove contaminating DNA. Reverse transcription was primed with Oligo(dt) primers and reaction incubated at 42 °C for 40 min and 15 min at 70 °C. Reverse transcription-PCR (RT-PCR) was performed to identify the endogenous expression of pluripotency markers (Sox2, Oct3/4 and Nanog) in iPSCs and the MN specific marker ChAT in iPSCs-derived MNs. The sequences of the primers are reported in Table 2.

2.7. Immunofluorescence

Cells were fixed in 4% paraformaldehyde for 20 min at room temperature. Permeabilization was performed using 0.3% Triton X-100 and ice-cold 100% methanol for nuclear staining. In order to avoid non-specific binding of antibodies, fixed cells were incubated for 20 min in a blocking solution (10% NGS in PBS) and thereafter, primary antibodies, diluted in blocking solution, were added for 90 min at 37 °C. Coverslips were then washed twice with the blocking solution and cells stained with Alexa 488 and Alexa 555 conjugated secondary antibodies for 45 min at room temperature. For nuclei staining, 4′,6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI) 2 µg/ml was finally added for 5 min at room temperature. Coverslips were rinsed with PBS, mounted with FluorSave (Merck, Darmstadt, Germany) and images acquired at confocal microscope (Nikon Eclipse C1, Minato, Japan). Primary antibodies used are listed in Table 3.

3. Results

3.1. Generation of iPSCs from peripheral blood cells

Cellular reprogramming of peripheral blood samples from the ALS patient (see Table1 for clinical characteristics) and the healthy control was performed using the integration-free CytoTune-iPS Sendai Reprogramming Kit. Three weeks after transduction, iPSC colonies were grown to an appropriate size for transfer. The number of emerged colonies was higher than 30 colonies/dish for both patient and control sample. At least 6 colonies were individually picked and transferred onto Geltrex coated dishes. Colonies were expanded for six passages before characterization, differentiation and freezing.

Table 3
Immunocytochemistry primary antibodies for pluripotency and differentiations.

Primary antibody	Supplier
Tra-1-60 (Podocalyxin) (TRA-1-60)	Invitrogen (Carlsbad, CA, USA)
SSEA4 (eBioMC-813-70 (MC-813-70))	Invitrogen (Carlsbad, CA, USA)
Alpha-fetoprotein (AFP)	Invitrogen (Carlsbad, CA, USA)
Alkaline phosphatase (AP)	Abcam (Cambridge, UK)
Desmin (D33)	DAKO (Santa Clara, CA, USA)
βIII tubulin (EP1569Y)	Abcam (Cambridge, UK)
MNR2/HB9/Mnx1 (81.5C10)	DSHB (Iowa City, IA, USA)
Pan-axonal neurofilament marker (SMI312)	Covance (Princeton, NJ, USA)
TDP-43	Proteintech (Rosemont, IL, USA)

3.2. Characterization and pluripotency of iPSCs

Cell morphology was typical of iPSCs, with a high nuclear to cytoplasmic ratio, forming compact multilayer colonies with defined edges. Their growth rate and morphology remained unchanged after several passages. One clone from both mutant TDP-43 ALS patient and control was further expanded and used for all the experiments. Genotyping confirmed mutation in the *TARDBP* iPSC line (Supplementary Fig. 1 a) and both clones had a normal karyotype (Supplementary Fig. 1 b–c). Both clones were positive for the expression of the widely used pluripotency markers SSEA4, Tra-1-60 and Alkaline Phosphatase (AP) as demonstrated by immunofluorescence (Fig. 1a) and Sox2, Oct3/4 and Nanog as shown by RT-PCR (Fig. 1b). The pluripotency of the two iPS cell lines was further assessed through the formation of EBs and subsequent spontaneous differentiation into the three germ layers. iPSC *in vitro* differentiation ability was evaluated by immunofluorescence, showing the expression of AFP (endoderm), βIII tubulin (ectoderm) and desmin (mesoderm) markers (Fig. 1c).

3.3. Motor neuron differentiation

Dissociated EBs plated on poly-lysine/laminin-coated coverslips were cultured for 10 days to allow MN differentiation (Fig. 2a). Most of the cells exhibited a typical neuronal morphology with long and abundant axons as shown by βIII tubulin staining (Fig. 2b). Expression of the MN specific marker HB9 was 15.5% in mutant TDP-43 patient-derived MNs and 20.4% in control cells (Fig. 2b–d). In both samples, 80% of the cells were positive for the anti-neurofilament antibody SMI312 (Fig. 2c). Moreover, expression of the Choline acetyltransferase (ChAT) enzyme was detected by RT-PCR in differentiated MNs from both ALS patient and healthy donor (Fig. 2e).

3.4. Analysis of TDP-43 sub-cellular localization in iPSCs and iPSCs-derived MNs

The RNA-binding protein TDP-43 is ubiquitously expressed and primarily localizes in the nucleus. We first examined TDP-43 sub-cellular localization in the mutant *TARDBP* p.A382T and control iPSCs. Our results demonstrated a prevalent nuclear distribution of TDP-43 in

Table 2
Primer sequences for pluripotency detection.

Gene	Forward primer	Reverse primer	Size (bp)	Ref.
Sox2	TTGCGTGAGTGTGGATGGGATTGGTG	GGGAAATGGGAGGGGTGCAAAA GAGG	151	Takahashi et al., 2007
Oct3/4	GACAGGGGGAGGGGAGGAGCT AGG	CTTCCCTCCAACCAGTTGCCCCA AAC	144	Takahashi et al., 2007
Nanog	CAGCCCTGATTCTTCCACCAGTCC	GTTCTGGAACCAGGTCTTCACCT G	244	-
ChAT	TGAGTACTGGCTGAATGACATG	AGTACACCAGAGATGAGGCT	144	Takahashi et al., 2007

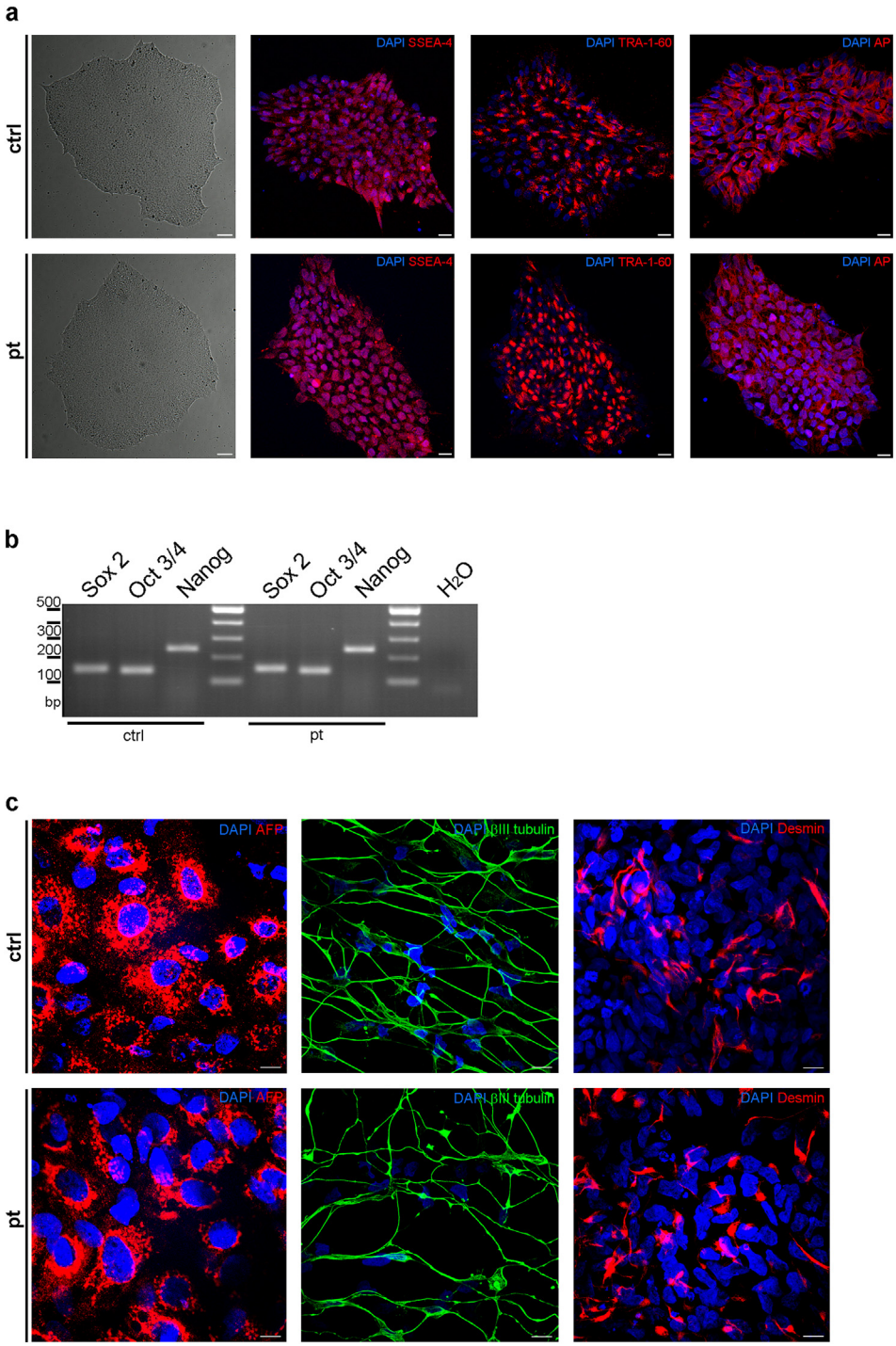

Fig. 1. Characterization of human iPSCs derived from peripheral blood mononuclear cells of a control (ctrl) and a mutated ALS *TARDBP* p.A382T patient (pt). (a) Left panels are representative pictures of iPSC colonies. Scale bar, 100 μm. Immunostaining showing positivity for the pluripotency markers SSEA-4, TRA-1-60 and AP. Nuclei are stained with DAPI. Scale bar, 20 μm. (b) RT-PCR showing the expression of pluripotency-associated genes Sox2, Oct3/4 and Nanog. (c) *In vitro* iPSCs differentiation displaying immunoreactivity for endodermal (AFP), ectodermal (βIII tubulin) and mesodermal (desmin) germ layer markers. Nuclei are stained with DAPI. Scale bar, 10 μm.

both patient and control iPSCs (Fig. 3a). As the neuropathological hallmark of ALS is the cytoplasmic mislocalization and aggregation of TDP-43 and its consequent nuclear depletion in disease-affected cells, we then investigated by immunofluorescence whether iPSCs-derived MNs from the patient displayed some of these pathological features. No significant difference was observed between the mutant *TARDBP* patient and the control MNs with most of the cells displaying a nuclear localization of TDP-43 protein (Fig. 3b). A very small number of cells presented evident cytoplasmic TDP-43 mislocalization in both samples (2,1% in control MNs vs 2,9% in ALS patient MNs), while aggregates were never observed (Fig. 3c).

4. Discussion

Primary causes and pathogenetic mechanisms of ALS are still mostly unknown and adequate experimental systems are highly needed to better understand the pathophysiology of this devastating disease. Animal models and cell lines currently available have allowed to only partially clarify the etiology of ALS and, clearly, the key issue has always been the difficulty to study the affected tissues. Indeed, until 2007 human MNs could only be obtained in culture from spinal cord explants (Quinn and De Boni, 1991) and dissociated cultures (Erkman et al., 1989). Human MNs have been successfully isolated by immunomagnetic beads (Silani et al., 1998) with limited expansion and survival capability. Moreover, spinal cord explant from living subjects

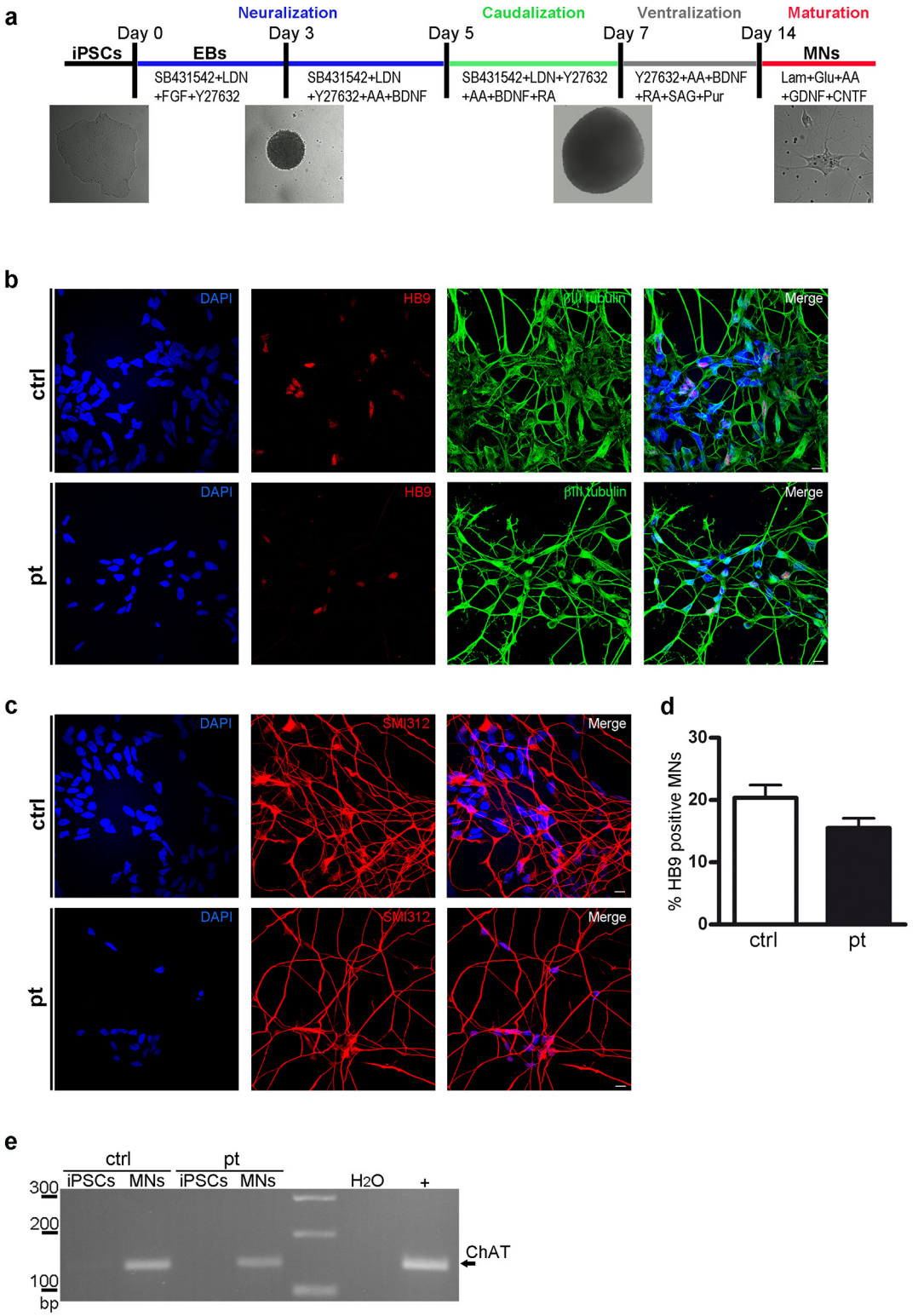

Fig. 2. MN differentiation of control (ctrl) and ALS patient iPSCs (pt). (a) Schematic representation of MN differentiation protocol from iPSCs. Immunocytochemical analysis of (b) HB9 (red) and βIII tubulin (green), and (c) SMI312 (red) ten days after EBs dissociation. Nuclei are stained with DAPI (blue). Scale bar, 10 μm. (d) Quantitative analysis of HB9 positive cells. (e) RT-PCR showing the expression of the motor neuronal marker ChAT. Commercial human MNs were used as positive control. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

requires invasive surgical procedures hardly applicable in humans. Thanks to the discovery of iPSCs by Takahashi et al. (Takahashi and Yamanaka, 2006), (Takahashi et al., 2007), human MNs can now be easily obtained potentially in unlimited quantity.

The aim of the present study is to report, for the first time, iPSCs reprogramming of peripheral blood mononuclear cells (PBMCs) of an ALS patient carrying the *TARDBP* p.A382T mutation, and their successful differentiation into MNs. Several authors have previously reported that fibroblast-derived iPSCs from both sporadic and familial ALS patients may be differentiated into MNs displaying a disease-

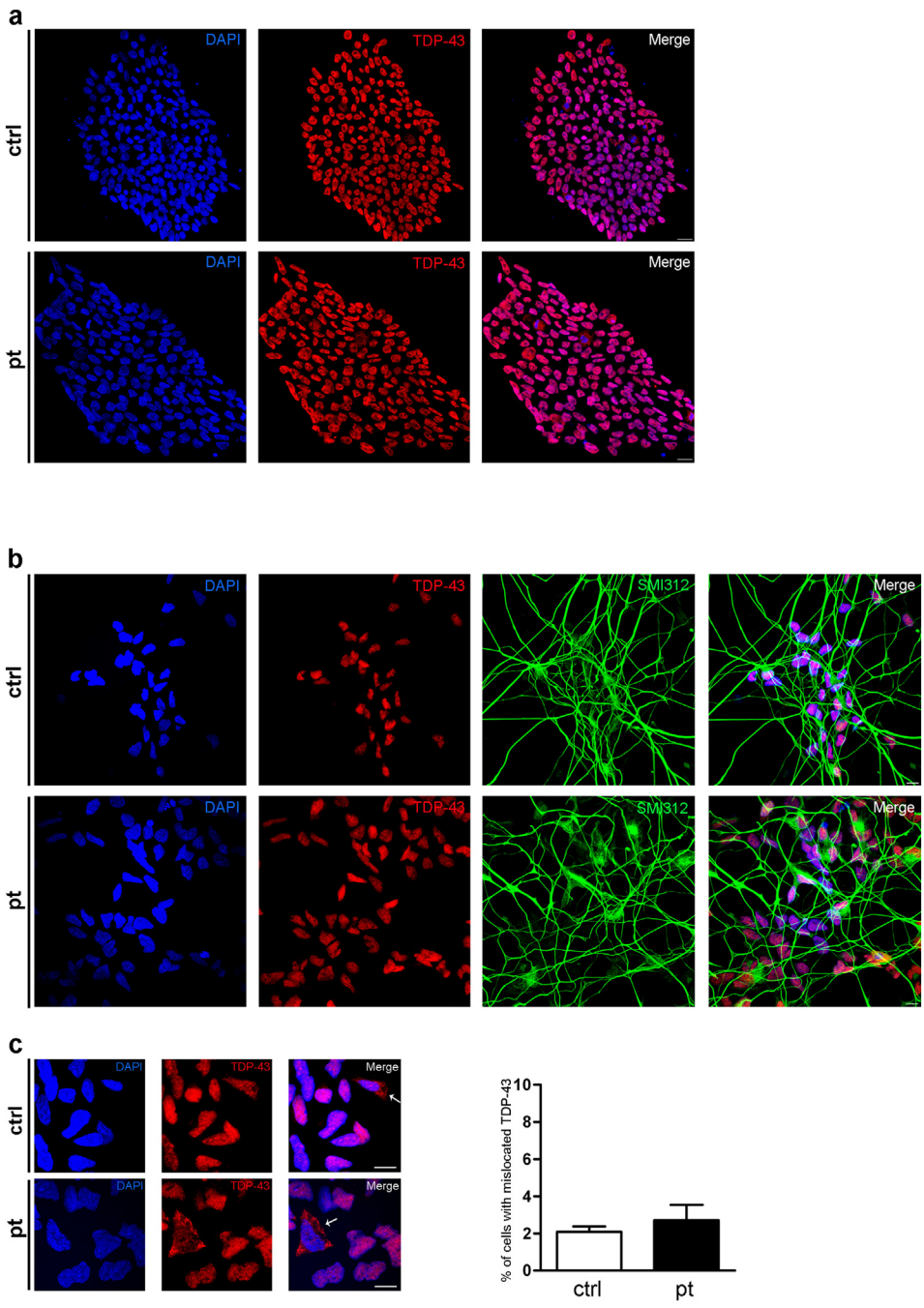

Fig. 3. TDP-43 expression in control (ctrl) and patient (pt) iPSCs and MNs. (a) Immunostaining of iPSCs showing TDP-43 nuclear expression. Nuclei are stained with DAPI. Scale bar, 20 μm. (b) Immunostaining of MNs with TDP-43 (red) and SMI312 (green). SMI312 staining was performed in order to visualize neuronal differentiated cells. Nuclei are stained with DAPI. Scale bar, 10 μm. TDP-43 localization was mainly nuclear, except for some cells with cytoplasmic positivity, as indicated by arrows (c). The histogram shows a quantitative analysis of the number of cells with TDP-43 mislocalization in the cytoplasm. For each sample, ten microscope fields displaying an average of 100 cells/field were analyzed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

specific phenotype (Almeida et al., 2013; Alves et al., 2015; Bilican et al., 2012; Burkhardt et al., 2013; Dimos et al., 2008; Donnelly et al., 2013; Egawa et al., 2012; Sareen et al., 2013). In addition, *TARDBP* p.M337 V mutation seems not to affect differentiation and maturation of functional MNs from fibroblast-derived iPSCs (Devlin et al., 2015). So far differentiation of MNs from PBMC-derived iPSCs has only been reported for a familial ALS patient carrying a *FUS* p.P525L mutation (Liu et al., 2015) and cytoplasmic mislocalization as well as FUS protein aggregation were observed.

In this study, we generated iPSCs starting from the PBMCs of an ALS patient and we compared their characteristics to PBMCs-derived iPSCs of a healthy donor as control. By using a Sendai reprogramming technology based on vectors expressing key genetic factors we have already obtained several iPSC lines with high efficiency from fibroblasts of both ALS patients and healthy donors (data not shown). Moreover, this reprogramming methodology is safe as Sendai vectors do not integrate into the host genome reducing the risk of genetic alterations in the host cell (Seki et al., 2010). In order to fulfil clinical application criteria and to maintain chromosome stability overtime, we grew iPSCs in a feeder-free commercial medium able to avoid animal-derived constituents and to perform long-term and stable serial culture passages without generating chromosomal abnormalities (Nakagawa et al., 2014).

We were able to obtain from both our peripheral blood samples stable iPSC lines that were fully characterized. The morphology of the iPSCs obtained was similar and displayed comparable expression of the pluripotency-related markers (SSEA4, Tra-1-60, AP, Sox2, Oct3/4 and Nanog). Similarly, no differences were observed concerning the spontaneous differentiation into the three germ lines indicating that the presence of the *TARDBP* p.A382T mutation did not interfere with pluripotency of the cells. iPSCs were then differentiated into MNs. When analyzed by immunofluorescence, no differences were observed for the expression of the axonal neurofilament marker SMI312 between

ALS patient and control iPSCs-derived MNs. Some variability was detected in MN differentiation outcomes with little different percentage of HB9 positive cells. Indeed, the TDP-43 mutated patient and the healthy control displayed 15.5% and 20.4% of HB9 positive cells, respectively. Different rates of differentiation have already been reported (Liu et al., 2015), (Mitne-Neto et al., 2011) and it has been speculated that patient age and different mutations may influence the percentage of fully differentiated MNs (Liu et al., 2015). In our study, the slight discrepancy may be explained by a random effect or by the different age of the subjects as the control was 14 years younger than the patient.

We finally investigated whether the mutant MNs recapitulate *in vitro*, without additional stressors, key aspects of ALS such as TDP-43 protein mislocation and aggregation. We found that TDP-43 localization was predominantly nuclear and only a very small percentage of cells displayed a cytoplasmatic staining with no aggregates and no differences between the mutant and wild-type. The physiological levels of cytoplasmic localization of the TDP-43 protein was 2,1% in control *vs* 2,9% in ALS patient. Previous studies reported a cell autonomous pathological phenotype of *TARDBP* mutated MNs differentiated from fibroblast-derived iPSCs. In particular, in MNs derived from a *TARDBP* p.M337 V mutated patient, predominantly nuclear TDP-43 was observed, albeit punctate TDP-43 staining in the soma and cell processes was frequently observed (Bilican et al., 2012). In another study, MNs were differentiated from iPSCs of ALS patients carrying p.Q343R, p.M337 V, or p.G298S TDP-43 mutations and the authors demonstrated that TDP-43 was distributed in both the nucleus and the cytoplasm, with TDP-43 forming preinclusion-like aggregates in the cytoplasm (Egawa et al., 2012). Our different findings may be explained by a clonal or patient-to-patient variability, or by the different mutation considered. More recently in agreement with our results, no difference was observed in the number of cytoplasmic puncta in MNs derived from *TARDBP* p.M337 V mutated patient compared to a control (Seminary et al., 2018) and no TDP43 mislocalization or aggregation formation was reported in iPSCs-derived MNs from a p.G294 V mutated patient (Kreiter et al., 2018). Further investigations with a higher number of patients carrying the p.A382T mutation and eventually other mutations are required to confirm our result.

In conclusion, we established integration-free iPSCs from the peripheral blood of an ALS patient carrying the *TARDBP* p.A382T mutation. We demonstrated that these cells were morphologically and phenotypically comparable to those obtained from a healthy donor, displaying the same ability to differentiate into MNs. In addition, no TDP-43 aggregation or abnormal cytoplasmic distribution were observed. Given the easy access of peripheral blood, iPSCs-derived MNs may be potentially generated from any patient and the establishment of an ALS-MNs cell bank will greatly help to explore the variety of pathogenic mechanisms of the disease.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.05.009.

Acknowledgements

This work has been supported by Fondazione Regionale per la Ricerca Biomedica (Regione Lombardia), project nr. 2015-0023. VG was supported by the Doctorate School of Molecular Medicine, Università degli Studi di Milano. We are grateful to Dr. A. Ratti and Dr. N. Ticozzi for critical revision of the manuscript.

Authors' contributions

All persons who meet authorship criteria are listed as authors.

PB and FS designed this study.

FS, PB and VG carried out the experiments, developed the methodology and analyzed the results.

SP performed the genotyping and GM the karyotypes.

VS provided the samples, the clinical data and supervised the all study.

PB and FS wrote the manuscript.

All authors contributed to and approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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