Transplanted motoneurons derived from human induced pluripotent stem cells form functional connections with target muscle

Huanxing Su, Lihui Wang, Jinglei Cai, Qiuju Yuan, Xiaoying Yang, Xiaoli Yao, Wai-Man Wong, Wenhao Huang, Zhiyuan Li, Jian-Bo Wan, Yitao Wang, Duanqing Pei, Kwok-Fai So, Dajiang Qin, Wutian Wu

Abstract

Induced pluripotent stem cells (iPSCs) hold promise for the treatment of motoneuron diseases because of their distinct features including pluripotency, self-derivation and potential ability to differentiate into motoneurons. However, it is still unknown whether human iPS-derived motoneurons can functionally innervate target muscles in vivo, which is the definitive sign of successful cell therapy for motoneuron diseases. In the present study, we demonstrated that human iPSCs derived from mesenchymal cells of the umbilical cord possessed a high yield in neural differentiation. Using a chemically-defined in vitro system, human iPSCs efficiently differentiated into motoneurons which displayed typical morphology, expressed specific molecules, and generated repetitive trains of action potentials. When transplanted into the...
Induced pluripotent stem cells (iPSCs) are promising for the treatment of neurological diseases because of their fascinating features: they are pluripotent, self-derived, and easily obtainable (Takahashi and Yamanaka, 2006; Yu et al., 2007). Many studies have shown that human iPSCs can successfully differentiate into functional neuronal subtypes such as dopaminergic neurons (Hargus et al., 2010; Rhee et al., 2011; Soldner et al., 2009) and motoneurons (Dimos et al., 2008; Hu et al., 2010; Karumbayaram et al., 2009), opening an avenue for clinical application of iPSC technology in the near future. Motoneuron diseases are characterized by degeneration of motoneurons, clinically manifesting with progressive paralysis and muscle wasting. There are currently no efficacious treatments for these diseases. Recently cell replacement strategies have been applied to treat neurological disorders with motoneuron degeneration (Corti et al., 2008, 2010; Deshpande et al., 2006; Gao et al., 2005). Grafted mouse embryonic stem cell (ESC)-derived motoneurons are reported to survive, extend axons into ventral roots, and promote functional recovery in rodent models of motoneuron degeneration, initiating hopes of successful cell replacement for degenerating motoneurons in the spinal cord (Corti et al., 2009; Deshpande et al., 2006; Harper et al., 2004). However, ethical concerns and immune rejection greatly hamper the clinical application of ESCs as sources for cell therapy (Testa et al., 2007; Lui et al., 2009).

Human iPSCs may be an appealing cell source for treating motoneuron diseases. They are reprogrammed to an embryonic state from human adult somatic cells (such as skin fibroblasts) by introducing pluripotency factors (Takahashi et al., 2007; Yu et al., 2007), which provides the opportunity for cell replacement therapy without ethical conflicts and requiring immunosuppressive therapy to overcome immune rejection. Recent studies have reported that human iPSCs can successfully differentiate into functional motoneurons using a chemically-defined in vitro system (Dimos et al., 2008; Ebert et al., 2009; Hu and Zhang, 2010; Karumbayaram et al., 2009), suggesting their therapeutic potential. However, a number of fundamental questions remain unanswered: whether iPSC-derived neurons can survive and integrate into the host and, more importantly, whether they can functionally innervate their targets after transplantation in vivo. In addition, it remains to be elucidated whether there are any potential risks associated with transplantation of iPSC-derivatives in vivo such as carcinogenesis resulting either from overexpression of an oncogene or through insertional mutagenesis into the host genome.

Using the rat model of brachial plexus injury, we investigated the survival, axonal extension and functional innervation of human iPSC-derived motoneurons after direct transplantation into the rat musculocutaneous nerve following brachial plexus injury. The results of the study provide evidence for the potential of iPSC-based therapies for the treatment of motoneuron diseases.
induction of motoneuron progenitors that were identified by Olig2 expression (day 21). For differentiation of post-mitotic motoneurons, the Olig2-expressing progenitor clusters were grown on laminin substrate. Neurotrophic factors, brain-derived neurotrophic factor (BDNF, 20 ng/ml), glial-derived neurotrophic factor (GDNF, 20 ng/ml), ciliary neurotrophic factors (CNTF, 20 ng/ml), insulin-like growth factor-1 (IGF1, 10 ng/ml), and cAMP (1 μM) were added to support the survival and process outgrowth of motoneurons.

Fluorescence-activated cell sorting

To quantify the neural differentiation potential of human iPSCs, cell clusters at day 14 and day 21 were fixed directly for fluorescence-activated cell sorting (FACS) analysis. After digestion with Accutase (Innovative Cell Technologies Inc., San Diego), cell clusters were dissociated to single cells, and washed with FACS buffer. After being fixed and permeabilized with ice-cold 0.1% paraformaldehyde for 10 min and 90% methanol for 30 min, cells from cell clusters at day 14 were incubated in primary antibody (Pax6, mouse IgG; 1:5000) and cells from cell clusters at day 21 were incubated in primary antibody (Olig2, goat IgG; 1:1000). Cells were then washed and incubated with the corresponding secondary antibody, FITC-conjugated anti-mouse or anti-goat IgG, for 2 h followed by washing steps. Cells were analyzed using a Becton Dickinson FACSCalibur instrument and CellQuest Pro software (BD Biosciences, San Diego).

Immunocytochemistry and quantification

Immunocytochemical staining on coverslip cultures and quantification were performed as previously described (Lee et al., 2007; Su et al., 2009) and primary antibodies were listed in Table 1. Species-specific secondary antibodies conjugated to the fluorescent labels Alexa 568 or 488 (1:400; Molecular Probes) were used to visualize primary antibodies. Cells were mounted in anti-fade medium containing 4′,6-diamidino-2-phenylindole (DAPI, Sigma) to counterstain the whole survival period.

Animal surgery and cell transplantation

Adult female Sprague-Dawley rats (220–250 g) were used. After anesthesia with intraperitoneal injection of ketamine (80 mg/kg) and xylazine (8 mg/kg), the right C5, C6 and C7 root avulsion was performed to detach the musculocutaneous nerve from the spinal cord. Immediately after root avulsion, animals received either cell transplantation (n = 12) or vehicle injection (n = 12). For cell preparation, neural rosettes after 14 days of differentiation were gently blown off and suspended in the neural medium. RA (0.1 μM) and sonic hedgehog (SHH, 200 ng/ml) were used for inducing the ventral spinal progenitor fate for 1 week, leading to an efficient induction of motoneuron progenitors that were identified by Olig2 expression. Suspended cell clusters after treatment with RA and SHH at day 21 were dissociated with Accutase into single cells and re-suspended in NB medium with 20 μg/ml BDNF, 20 μg/ml GDNF, 20 μg/ml CNTF and 10 μg/ml IGF at a concentration of 1.0 × 105 cells/μl, and placed on ice for the duration of the grafting session. Cell viability was assessed with trypan blue at the end of transplantation and typically over 90% of the cells excluded the dye. One microliter cell suspension or vehicle (NB medium plus the cocktail of neurotrophic factors described above) was slowly injected into the right musculocutaneous nerve. After cell injection, the proximal end of the musculocutaneous nerve was ligated to confine the injectant. Animals were allowed to survive for 16 weeks (12 rats per group). Cyclosporin A (10 mg/kg, Sigma) was injected intramuscularly daily to suppress immune rejection during the whole survival period.

Electrophysiological analysis

Whole-cell patch-clamp recordings

Whole-cell patch-clamp recording techniques were used to study the intrinsic properties of umbilical cord-iPSC-derived

---

**Table 1** Primary antibody list.

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Isotype</th>
<th>Dilution</th>
<th>Source</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pax6</td>
<td>Mouse IgG</td>
<td>1:2,000</td>
<td>Developmental Studies Hybridoma Bank</td>
<td>Pax6</td>
</tr>
<tr>
<td>SOX1</td>
<td>Goat IgG</td>
<td>1:1,000</td>
<td>R&amp;D Systems</td>
<td>AF3366</td>
</tr>
<tr>
<td>Nestin</td>
<td>Rabbit IgG</td>
<td>1:1,000</td>
<td>Sigma</td>
<td>NS413</td>
</tr>
<tr>
<td>Olig2</td>
<td>Goat IgG</td>
<td>1:500</td>
<td>Santa Cruz Biotechnology</td>
<td>SC-19669</td>
</tr>
<tr>
<td>HoxB4</td>
<td>Rat IgG</td>
<td>1:50</td>
<td>Developmental Studies Hybridoma Bank</td>
<td>112 anti-Hoxb4</td>
</tr>
<tr>
<td>Islet1</td>
<td>Mouse IgG</td>
<td>1:400</td>
<td>Developmental Studies Hybridoma Bank</td>
<td>40.3A4</td>
</tr>
<tr>
<td>hNluclei</td>
<td>Mouse IgG</td>
<td>1:200</td>
<td>Chemicon</td>
<td>MAB1281</td>
</tr>
<tr>
<td>Lhx3</td>
<td>Mouse IgG</td>
<td>1:500</td>
<td>Developmental Studies Hybridoma Bank</td>
<td>67.4E12</td>
</tr>
<tr>
<td>Hb9</td>
<td>Mouse IgG</td>
<td>1:50</td>
<td>Developmental Studies Hybridoma Bank</td>
<td>81.5C10</td>
</tr>
<tr>
<td>ChAT</td>
<td>Goat IgG</td>
<td>1:200</td>
<td>Chemicon</td>
<td>AB144P</td>
</tr>
<tr>
<td>hNF70</td>
<td>Mouse IgG</td>
<td>1:200</td>
<td>Chemicon</td>
<td>MABS294</td>
</tr>
<tr>
<td>Tuj1</td>
<td>Rabbit IgG</td>
<td>1:5,000</td>
<td>Covance</td>
<td>PRB-435P</td>
</tr>
<tr>
<td>α-BT</td>
<td>NA</td>
<td>1:500</td>
<td>Molecular Probe</td>
<td>B13423</td>
</tr>
</tbody>
</table>
motoneurons in culture. Patch pipettes (resistance 3–5 MΩ) were filled with the following (in mM): 140 potassium methanesulfonate, 5 NaCl, 1 CaCl₂, 0.2 EGTA, 3 ATP-Na₂, 0.4 GTP- Na₂, pH 7.3 (adjusted with KOH). The external solution contained (in mM): 120 NaCl, 1.2 KH₂PO₄, 1.9 KCl, 26 NaHCO₃, 2.2 CaCl₂, 1.4 MgSO₄, 10 d-glucose, 7.5 HEPES (pH with NaOH to 7.3). The bath solution was equilibrated with 95% O₂ and 5% CO₂ before use. Resting potentials were maintained at about −65 mV. Whole-cell patch-clamp recordings were amplified and filtered using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA). Signals were sampled at 10 kHz using a Digidata 1440A analog-to-digital converter and acquired and stored on a computer hard drive using pClamp10 software. All voltage and current-clamp recordings were performed as described previously (Miles et al., 2004). Data were analyzed using pClamp10 (Clampfit).

Electromyographic measurement (EMG)

At the end of the study, animals were killed, and the musculocutaneous nerve and biceps brachii muscle were quickly removed and transferred into a recording chamber that was continuously perfused with room-temperature oxygenated mouse Tyrode’s solution (in mM: 125 NaCl, 24 NaHCO₃, 5.4 KCl, 1 MgCl₂, 1.8 CaCl₂, and 5% dextrose) at a flow rate of 4–5 ml/min. EMG recordings were obtained using a suction electrode on the surface of the muscle while a second suction electrode was used to stimulate the nerve. EMG signals were amplified with a differential amplifier (EX4-400, Dagan) and captured using Axoscope 9.2 software (Molecular Devices).

Tissue analysis

The biceps brachii muscle was weighed after EMG recording. The musculocutaneous nerve and biceps brachii were then fixed in 3.7% formaldehyde and cryo-sectioned at 30 μm. Longitudinal nerve sections were incubated with the primary antibodies including human Nuclei, human-specific neurofilament 70, Hb9 and ChAT to indentify the survival, maturation and axonal extension of transplanted human iPSC-derived motoneurons. The total number of human Nuclei-expressing cells was counted in every fourth section. To detect phenotypes of transplanted cells, we stained three tissue sections per rat for each neuronal marker and then counted the number of the cells with the positive staining of the neuronal marker in 10 random fields per section at 40× magnification using the confocal microscope. All quantification was performed in an unbiased stereological manner. Biceps brachii muscle sections were incubated with

Fig. 1  Hi-UMC exhibited high efficiency in neural differentiation. A–C: Human ESCs grew as individual colonies (A), formed neural tube-like rosettes at day 14 (B), and expressed the neural epithelial marker (NE) Pax6 (C). D–F: Human iPSCs grew as individual colonies (D), formed neural tube-like rosettes at day 14 (E), and expressed Pax6 (F). G and H: At day 14, FACS analyses revealed that both hi-UMC and H9 exhibited a high efficiency in generating Pax6-expressing NE cells (64.8 ± 7.5% and 85.3 ± 6.1% respectively). Scale bar: 100 μm for A, B, D, and E; 25 μm for C and F.
1 μg/ml of rhodamine-conjugated α-bungarotoxin (Molecular Probes) for 1 h at room temperature and double-labeled with human-specific neurofilament 70 to visualize transplanted human cell-derived acetylcholine receptors (AChRs).

Statistical analysis

Data are expressed as mean ± SEM. Student's two-tailed t-test was used for comparison of two experimental groups. Changes were identified as significant if p was less than 0.05.

Results

Hi-UMC showed high efficiency in neural differentiation and generated functional motoneurons in vitro

A schematic illustration was used to show our experimental design (Fig. S1). Using a well-established protocol (Hu and Zhang, 2009; Li et al., 2005), we firstly investigated the neural differentiation capacity of hi-UMC which was derived from mesenchymal cells of the umbilical cord (Cai et al., 2010). Human iPSCs were cultured in ESC medium on a feeder layer of irradiated embryonic mouse fibroblasts. They grew as round colonies and were morphologically indistinguishable from human ESCs, such as exhibiting a large nucleus and multiple, prominent nucleoli (Figs. 1A and D). By 10–14 days differentiation, both human ESCs and iPSCs successfully generated columnar neuroepithelial cells and formed typical neural tube-like rosettes (Figs. 1B and E). Immunostaining showed that the columnar cells in the rosettes expressed early neural epithelial (NE) marker Pax6 (Figs. 1C and F). FACS analyses demonstrated that both hi-UMC and H9 exhibited high efficiency in generating Pax6-expressing NE cells (64.8 ± 7.5% and 85.3 ± 6.1% respectively) (Figs. 1G and H).

We then investigated motoneuron differentiation of hi-UMC. By 10 days differentiation, typical neural tube-like rosettes were formed (Fig. 2A). Cells in the rosettes became positive for Sox1 and a small proportion were positive for Nestin (Fig. 2B). We therefore treated the NE cells with RA at day 10 for motoneuron specification. Four days later, many cells were found to express HOXB4, suggesting that the primitive NE cells were induced into progenitors with spinal cord identity by RA treatment (Figs. 2D and K; 57.5 ± 4.7% of total Tuj1-positive cells). From day 15, after lifting off rosettes and treatment with RA and SHH for 1 week, an efficient induction of motoneuron progenitors identified by Olig2 expression was obtained (Fig. 2C). FACS analysis demonstrated that around 75.5 ± 8.1% cells were Olig2-positive (Fig. 2J). We replated these Olig2-expressing progenitor clusters onto laminin-coated dishes and added a cocktail of neurotrophic factors and cAMP to support their survival and outgrowth (Fig. 2E). These Olig2-expressing cells then differentiated into motoneurons in the 5th week and expressed Hb9 (Figs. 2F and K; 31.2 ± 5.6% of total Tuj1-positive cells) and Islet1 (Figs. 2G and K; 28.6 ± 3.6% of total Tuj1-positive cells). When further cultured in the presence of neurotrophic factors and cAMP, some cells became Lhx3-positive (Figs. 2H and K; 19.4 ± 2.7% of total Tuj1-positive cells). Expression of Lhx3 in motoneuron progeny suggests that a subset of hiPSC-derived motoneurons expressed medial motor column markers. Cells that expressed ChAT, a key enzyme catalyzing acetylcholine synthesis for signal conduction, appeared in the 5th week (3 weeks after the neuroectodermal cells were plated for motoneurons differentiation). The number of ChAT-positive cells increased steadily and reached a high level at 5 weeks after plating of the neuroectodermal cells (Figs. 2l and K; 64.5 ± 5.2% of total Tuj1-positive cells).

Standard whole-cell patch clamp recordings were then performed to investigate whether the molecular characteristics of iPSC-derived motoneurons were accompanied by the acquisition of functional properties typical of mature motoneurons (n = 25) (Fig. 3A). Voltage-clamp recordings demonstrated that most cells generated large, rapidly-inactivating inward currents in response to depolarizing voltage steps that were sensitive to TTX (10 μM) (Fig. 3B), and persistent outward currents activated by depolarizing steps from a holding potential of -100 mV that were sensitive to TEA (30 μM) (Fig. 3C). Using current-clamp recordings, roughly half of tested neurons generated repetitive trains of action potentials, suggesting that they were functional (Fig. 3D). All these data demonstrated that hi-UMC can successfully develop into phenotypically and physiologically mature motoneurons in vitro. After recordings were made, the neurons were fixed and double immunostaining with biocytin and ChAT demonstrated that many of the cells which we recorded were motoneurons (data not shown).

Transplanted hi-UMC-derived motoneurons survived and extended axons in the musculocutaneous nerve

These functional human iPSC-derived motoneurons are attractive for cell-based therapies for motoneuron diseases. However, whether they survive after transplantation in vivo, and more importantly, whether they can form functional connections with target muscles are critical for evaluating their therapeutic potential. Using the brachial plexus injury model, we transplanted hi-UMC-derived motoneuron progenitors directly into the musculocutaneous nerve to investigate their survival, maturation, axonal extension and functional innervation of the target muscles (Fig. 4A). Compared to the central nervous system, the peripheral nervous system is more favorable for cell survival, closer to the target muscles and less affected by endogenous neuronal circuits, thus facilitating assessment of the ability of grafted cells to survive and reinnervate their targets (Thomas et al., 2000; Yohn et al., 2008). By 16 weeks after transplantation, robust graft survival was observed in the musculocutaneous nerve as evidenced by immunohistochemistry for human Nuclei (Fig. 4B). Approximately 1.8 × 10⁴ human Nuclei-positive cells were found in the nerve, representing that 18% of initial grafted cells survived at 16 weeks after transplantation. We co-labeled these human Nuclei-positive cells with a human specific neuronal marker, human specific neurofilament 70, and found that around 71.5 ± 6.2% of them were human specific neurofilament 70-positive (Figs. 4B, b and C). Furthermore, immunostaining with ChAT and Hb9 showed that grafted hiPSC-derived cells could give rise to ChAT-positive and Hb9-positive motoneurons in the nerve (Figs. 5A, B, a and c).

We then examined axonal extension of graft-derived motoneuron-like cells in the musculocutaneous nerve. Double immunostaining with human specific neurofilament 70 and ChAT demonstrated that human specific neurofilament
70-positive axons were present throughout the transected nerve and clearly shown to be co-labeled with ChAT, suggesting they were extending from the cell graft towards the nerve terminal (Fig. 5b).

**Transplanted hi-UMC-derived motoneurons formed functional connections with the target muscle**

To determine whether the terminals of iPSC-derived motoneurons formed synaptic connections with the target muscle, anti-human-specific neurofilament 70 antibody was used to visualize human iPSC-derived motoneuron axons and rhodamine-conjugated α-bungarotoxin (BT) was used to label postsynaptic ACHRs at the motor endplates. Human-specific neurofilament-positive axons were in close apposition to the motor endplates at 16 weeks after transplantation (Fig. 6A), whereas no neuromuscular junctions were co-labeled with human-specific neurofilament antibody in vehicle control (Fig. 6B) and normal control rats (Fig. 6C). We then proceeded to stimulate the peripheral nerve in isolated nerve-muscle preparations to induce EMG responses. Successful EMG
responses were induced in all the animals with cell transplants (Fig. 6F1), while no EMG response was found in vehicle control animals at 12 weeks after injury (data not shown). To confirm that the muscle contractions resulted from neurotransmission by the transplanted iPSC-derived motoneurons, the competitive nicotinic antagonist d-tubocurarine was added to the recording solution. EMG activity was blocked by d-tubocurarine (Fig. 6F2) and this blockade was reversed after a 5-h washout period (Fig. 6F3). These data indicated functional innervation of biceps brachii by implanted iPSC-derived motoneurons. In cell implanted animals, significantly more neuromuscular junctions were found compared to vehicle control animals (Fig. 6D; 8.1 ± 2.4 vs 1.3 ± 1.2 per mm²; \( P < 0.05 \)) and the muscle wet weight of biceps brachii muscle from the cell implanted animals was nearly triple of that of vehicle control animals (Fig. 6E; 68.4 ± 12% of normal side vs 23.5 ± 6 of normal side; \( P < 0.001 \)), suggesting that transplanted cells efficiently protected against the muscle atrophy resulting from brachial plexus injury.

**Discussion**

Stem cells-based replacement strategies show a promising future for the treatment of motoneuron diseases due to their ability to replace lost, damaged or dysfunctional neurons. However, it still needs further studies to decide on which stem cell type is the optimal cell source for transplantation therapy. ESCs have been considered to be an ideal cell source of motoneurons. Both murine and human ESCs are able to differentiate into functional motoneurons when exposed to SHH and RA (Li et al., 2005; Wichterle et al., 2002). However, immune rejection and ethical controversy greatly hurdle the clinical application of ESCs.
iPSCs seem to be an optimal cell source for transplantation therapy. They have the advantage of eliminating ethical and immune rejection concerns. The generation of iPSCs from a patient's own somatic cells would potentially allow for a plentiful source of cell therapeutics for autotransplantation. A number of studies have reported that human iPSCs can successfully differentiate into motoneurons (Dimos et al., 2008; Ebert et al., 2009; Hu and Zhang, 2010; Karumbayaram et al., 2009). However, all these studies did not investigate the capacity of human iPSC-derived motoneurons for functional innervation after transplantation in vivo.

In the present study, we demonstrated that human iPSCs derived from mesenchymal cells of the umbilical cord possess high efficiency in neural differentiation and can efficiently differentiate into phenotypically and physiologically mature motoneurons in vitro. Using the model of the musculocutaneous nerve and its target muscle, our study investigated the survival and the capacity for functional innervation of human iPSC-derived motoneurons after transplantation in vivo. Our results showed that grafted human iPSC-derived motoneurons not only survive well in vivo but also are capable of forming functional connections with target muscles. More importantly, they significantly attenuated denervated muscle atrophy after transplantation into the peripheral nerve close to the target muscle, which may preserve endogenous motoneuron terminals and offer a possibility of later formation of functional connections between regenerating axons and denervated muscles. In our surgical paradigm, the proximal end of the musculocutaneous nerve was ligated to confine the injectant; thus transplanted cells were disconnected to the central nervous system. Using this paradigm, we answered the questions whether transplanted hiPSC-derived motoneurons could survive and innervate into the target muscle in vivo and demonstrated that hiPSC-derived motoneurons are truly functional not only in vitro but also in vivo, setting the basis for using hiPSC-derivatives to treat motoneuron diseases.

No host-derived endogenous nerve connection with the target muscle existed due to the ligation on the proximal end of the musculocutaneous nerve. Graft-derived innervation was revealed by staining with rhodamine-conjugated α-bungarotoxin and human-specific neurofilament 70. Nearly half of biceps brachii muscle fibers were innervated following transplantation of hiPSCs-derived motoneurons. The extent of reinnervation depended on the number of survived motoneurons, the number of graft-derived myelinated axons and functional motor units in the target muscle. Our study demonstrated that transplantation of $1.0 \times 10^5$ hiPSC-derived motoneurons results into significant re-innervation and functional recovery. However, it remains unknown whether transplantation of more neurons will lead to more innervation and better functional recovery. Future work is needed to explore the correlation between the timing of cell transplantation and the efficiency of axonal regeneration and neuromuscular junction formation and the correlation between the number of transplanted cells and the extent of reinnervation as well.

In summary, our study provides evidence for the first time on the in vivo survival and functional innervation of human iPSC-derived motoneurons, a key requirement in the development of iPSC-based cell therapy for motoneuron diseases. It should be noted, however, that future studies are essential regarding the capacity of human iPSC-derived
motoneurons for long-distance axonal growth and the ability to innervate appropriate peripheral targets. HiPSC-derived motoneurons will be transplanted into the ventral horn of the spinal cord of animals with motoneuron degeneration such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). We will investigate whether they can survive and integrate into the local circuit in the ventral horn, and whether they can find appropriate ways to grow axons to innervate target muscles and improve functional recovery. Results of these studies will help us fully address the therapeutic potential of hiPSCs in cell replacement strategies for the treatment with motoneuron degeneration diseases.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2013.02.007.

Acknowledgments

This study was supported by HK SCI Fund, Ministry of Science and Technology 973 program of China (2011CB504402 and 2012CB966802), the National Natural Science Foundation of China (81071901), the “Strategic Priority Research Program” of the Chinese Academy of Sciences, Grant No. XDA01020401, and the National S & T Major Special Project on Major New Drug Innovation, Grant No. 2011ZX09102-010-01. This study was also supported by a multi-year research grant from the University of Macau (MYRG122-ICMS12-SHX).
Fig. 6 Transplanted hi-UMC-derived motoneurons formed functional connections with the target muscle and reduced muscle atrophy. A: Axon terminals labeled with a hNF antibody and α-bungarotoxin (α-BT)-labeled neuromuscular junctions (NMJs) showing nerve terminals of human iPSC-derived motoneurons attached to NMJs. B and C: No NMJs were co-labeled with hNF in vehicle control (B) and normal rats (C). D: The number of NMJs in cell implanted animals (n = 12) was significantly higher than in vehicle control animals (n = 12) (8.1 ± 2.4 vs 1.3 ± 1.2 per mm²; P < 0.05). E: The wet weight of biceps brachii from the cell implanted animals was nearly triple of that in vehicle control animals (n = 12 in each group, P < 0.001). F: Successful EMG responses were induced in animals with cell transplants (F1); EMG responses were mediated through the NMJ, since application of α-tubocurarine effectively attenuated EMG activity (F2), which recovered after a 5-h washout period (F3). Scale bar: 120 μm for A, B and C.

References


Dimos, J.T., et al., 2008. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science 321 (5893), 1218–1221.


