Data in Brief 22 (2019) 199–203

Contents lists available at ScienceDirect

Data in Brief

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

Data Article

Data on in vivo PGC-1alpha overexpression model via local transfection in aged mouse muscle

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ARTICLE INFO

Article history:
Received 2 November 2018
Received in revised form
7 December 2018
Accepted 10 December 2018
Available online 13 December 2018

ABSTRACT

The data presented in this article are related to the research paper entitled "Intensified mitophagy in skeletal muscle with aging is downregulated by PGC-1alpha overexpression in vivo" (Yeo et al., 2019). The data explained the surgical procedure of in vivo local transfection by electroporation method in aged mouse tibialis anterior muscle, and plasmid DNA preparation and verification protocol. The data also showed the transfection efficiency levels of GFP or GFP-tagged PGC-1alpha through immunohistochemistry method for frozen muscle cross-sections.

Specifications table

Subject area	*Biology*
More specific subject area	*Skeletal muscle in vivo transfection*
Type of data	*Image*

DOI of original article: https://doi.org/10.1016/j.freeradbiomed.2018.10.456
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https://doi.org/10.1016/j.dib.2018.12.032

How data were acquired	*Fluorescence Microscope (Nikon Ti-S eclipse microscope with NIS-Elements 4.1.1 software)*
Data format	*Raw*
Experimental factors	*Fluorescence live cell imaging; C2C12 myoblast cells transfected with GFP-tagged plasmid DNA were cultured and imaged using microscope, immunohistochemistry; tibialis anterior (TA) muscle transfected with plasmid DNA was isolated, cross-sectioned, stained with appropriate antibodies and imaged using microscope.*
Experimental features	*Aged C57BL/6 J mice were anesthetized and transfected with GFP-tagged plasmid DNA in TA muscle via electroporation. After 5 days, TA muscle were collected and prepared for immunohistochemistry.*
Data source location	*Laboratory of Physiological Hygiene and Exercise Science, School of Kinesiology, University of Minnesota Twin Cities, Minneapolis, MN 55455, United States of America*
Data accessibility	*Data are with this article*
Related research article	*Yeo D, Kang C, Gomez-Cabrera MC, Vina J, Ji LL. Intensified mitophagy in skeletal muscle with aging is downregulated by PGC-1alpha over-expression in vivo, Free Radic Biol Med. 130, 2019, pp. 361–368* [1].

Value of the data

- The Establishment of an in vivo PGC-1alpha overexpression model of local muscle transfection using electroporation in aged mice indicates that this method can be applied to a wider range of possibilities to study aged mouse muscle.
- Providing the detailed experimental methodologies will help other researchers who want to develop their own model of muscle transfection.
- Valuable for researchers interested in the relationship between PGC-1alpha or other novel genes and sarcopenia.

1. Data

Here, we present data regarding PGC-1alpha in vivo transfection in aged mouse muscle [1]. The data demonstrated surgical procedures for in vivo transfection (Fig. 1); GFP or GFP-PGC-1α expression levels in C2C12 cell line for verifying plasmid DNA transfection efficiency (Fig. 2); and protein expression levels of GFP or GFP-PGC-1α in TA muscles, and the transfection efficiency in both young and old mice using immunohistochemistry (Fig. 3).

2. Experimental design, materials and methods

2.1. Animals and experimental group

Young (age 8–10 weeks) and old (age 24 month) female C57BL/6J mice were randomly divided into the following four groups: 1) GFP transfection in young (Y-GFP, $n = 7$); 2) PGC-1 transfection in young (Y-OE, $n = 7$); 3) GFP transfection in old (O-GFP, $n = 7$); 4) PGC-1 transfection in old (O-OE, $n = 7$). All surgical procedures and experiments protocol on mice were approved by Research Animal Resource Center at the University of Minnesota Twin Cities.

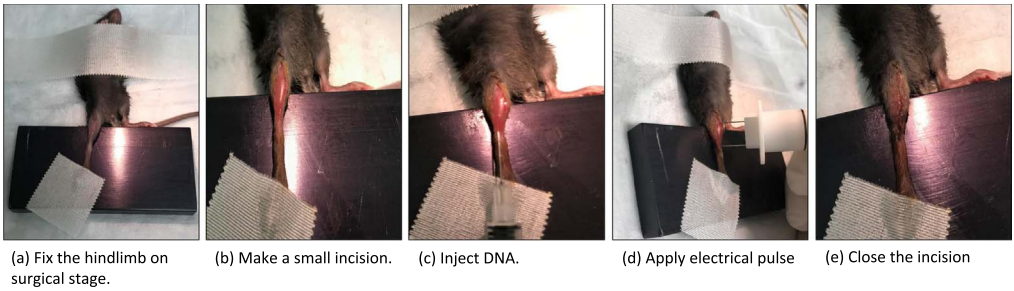

Fig. 1. Stepwise procedure of in vivo transfection. Once the mouse is completely anesthetized, shave one of the hindlimbs. Then, with the mouse lying on a supine position, pull the hindlimb and fix the foot on a small surgical stage. (b) Make a 1 cm small incision on the anterior aspect of the TA muscle by cutting through the skin without muscle damage. (c) Take the 10 μg of plasmid DNA using a 27-gauge insulin syringe. Insert the syringe needle no more than 3 mm along the longitudinal axis near the distal myotendinous junction of the TA muscle. Then, inject DNA into the muscle. Inject another 10 μg of plasmid DNA neat the proximal myotendinous junction of the TA muscle, using the same procedure described above. (d) Put the electrodes on the top of the proximal and distal myotendinous junctions of the TA muscle and apply 10 sq-wave electric pulses at a 180 V/cm. 20 ms, 1 Hz by using an electroporator. (e) Close the incision with Vetbond surgical glue and put the mouse on a heating pad to support for recovery from the anesthesia.

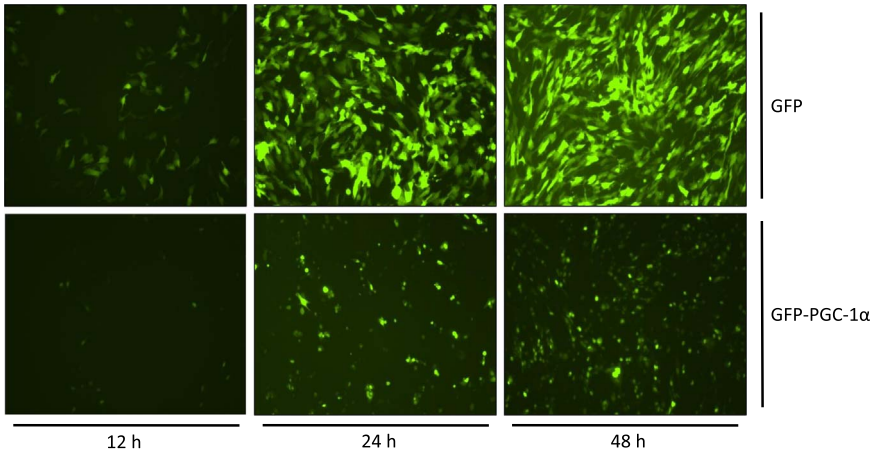

Fig. 2. Fluorescent images of overexpressed GFP and GFP-PGC-1α in C2C12 Myoblast. Seed C2C12 cells 40% confluent on 6 well cell culture plate, and incubate at 37 °C in 5% CO2 until the cells reach 70% of confluency. Transfected 2 ug of either GFP or GFP-PGC-1α DNA using Lipofectamine™ 2000 and incubate 4 h at 37 °C in 5% CO_2. Then, replace the media, and incubate 2 more days. By using fluorescence microscope equipped with FITC filters, visualizing and the transfected plasmid DNA and taking images by following time points: 12 h, 24 h, and 48 h, respectively.

2.2. Plasmid DNA preparation and verification

GFP-tagged PGC-1α plasmid was kindly provided from Dr. Bruce M. Spiegelman (Plasmid #4; Addgene, USA). Cloning of this plasmid DNA was performed via transformation with DH5α competent cells (Invitrogen, USA) following manufacturer's instruction. Extracting plasmid DNA from E. *coli* was carried out by plasmid megaprep kit (Qiagen, USA). Diagnostic restriction enzyme digest was carried out by using BamHI and Sall restriction enzymes (Invitrogen), respectively. Agarose gel electrophoresis was performed to confirm the plasmid fragments size after restriction enzyme digestion. Testing the plasmid DNA transfection efficiency was performed on C2C12 cell line using Lipofectamine™ 2000 transfection reagent (Invitrogen). Visualization of the transfected plasmid DNA was carried out via using fluorescence Microscope equipped with GFP or FITC filters (Fig. 2).

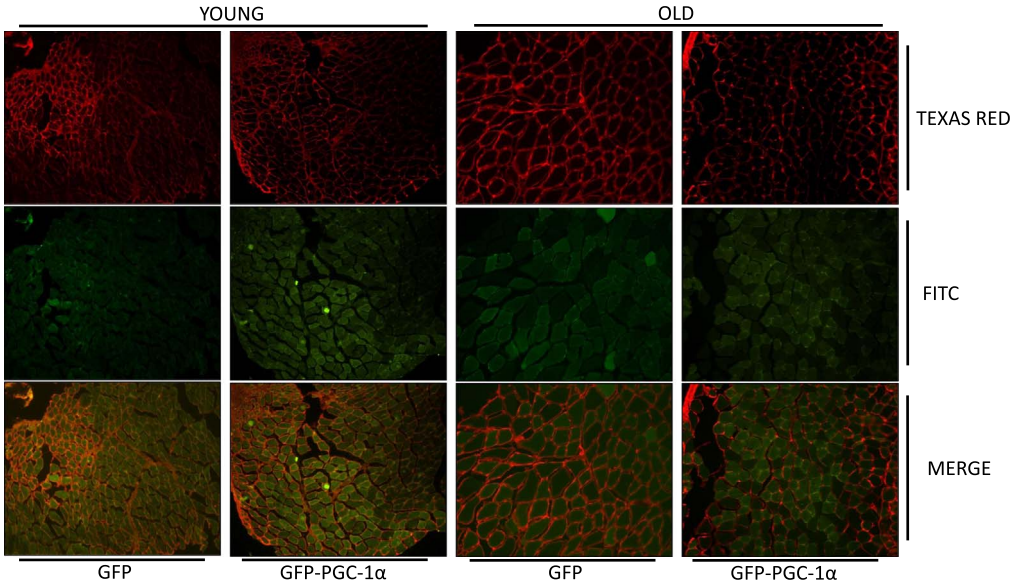

Fig. 3. Fluorescent images of mouse TA muscle cross-section expressing GFP and GFP-PGC-1α. Muscle cross-sections were incubated with anti-laminin antibody and Alexa Fluor 594 antibody which stained the muscle cell outer membrane. Visualize muscle sections using a fluorescence microscope with Texas Red and FITC filters.

2.3. *in vivo plasmid DNA transfection*

in vivo plasmid DNA transfection was carried out as previously described [2]. For anesthetizing a mouse, ketamine-xylazine cocktail (0.1 mL/20 g body weight) was administered by intraperitoneal injection, and a small incision was made through the skin where it covers the TA muscle. Injection of plasmid DNA (2.5 ug/ul GFP or 2.7 μg/μl GFP-PGC-1α) with 27-gauge insulin syringe was applied into the proximal (6 μL) and distal (6 μL) ends of the muscle belly. Electroporation was administered by two stainless steel pin electrodes (1 cm gap, BTX-Harvard Apparatus, USA) laid on top of the proximal and distal myotendinous junctions. Electric pulses were delivered with an ECM 830 electroporation unit (10 × 20 ms sq-wave length, 1 Hz frequency with 180 V/cm field strength, BTX-Harvard Apparatus). Then, the incision was closed with Vetbond surgical glue (3M, USA).

2.4. *Frozen muscle cryosectioning and immunohistochemistry*

Frozen TA muscle cross-sectioning and immunohistochemistry analysis were performed as previously described [3]. Briefly, 4% paraformaldehyde fixation and sucrose cryopreservation procedures applied to 5 days transfected TA muscle. Embed the TA muscle in Tissue-Tek OCT compound (Sakura Finetek, USA) and immediately freeze it with liquid nitrogen chilled isopentane. Cross sections (12 mm in thickness) from the mid-belly of the muscle. Sections were then washed in DPBS for 15 min followed by 20 min incubation in blocking solution (DPBS containing 0.5% bovine serum albumin and 0.5% Triton X-100). Sections were incubated with anti-laminin primary antibody (ab11575, Abcam, UK) for 1 h at room temperature. Sections were then washed with DPBS and incubate with the Alexa Fluor 594 conjugated secondary antibody (Invitrogen) for 1 h at room temperature. After that, the sections were washed and mounted with a coverslip. GFP-tagged plasmid DNA transfected fibers and laminin were identified in dual-fluorescent images by using Nikon Eclipse Ti-S fluorescent microscope and captured images with NIS-Elements 4.11 (Nikon, Japan).

Muscle cross-sections were incubated with anti-laminin antibody and Alexa Fluor 594 antibody which stained the muscle cell outer membrane. Visualize muscle sections using a fluorescence microscope with Texas Red and FITC filters.

Transparency document. Supporting information

Transparency document associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2018.12.032.

References

[1] D. Yeo, C. Kang, M.C. Gomez-Cabrera, J. Vina, L.L. Ji, Intensified mitophagy in skeletal muscle with aging is downregulated by PGC-1alpha overexpression in vivo, Free Radic. Biol. Med. 130 (2019) 361–368.
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