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Data Article

Data in support of protocol for rat single muscle-fiber isolation and culture

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ABSTRACT

This data article contains data related to the research article entitled, "Protocol for rat single muscle-fiber isolation and culture" by Komiya et al. [1]. It has yet to be shown whether adult myosin heavy chain (MyHC) isoforms are expressed at a readily detectable level in cultured myotubes. In this study, we examined whether adult MyHC isoforms could be detected in myotubes differentiated from rat satellite cells using a Western blotting assay and specific antibodies against slow MyHC, fast MyHC and pan-MyHC. Results showed that slow adult MyHC isoforms were faintly detected in myotubes, suggesting that rat myotubes express adult MyHC isoforms although that amount is very low.

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Specifications table

Subject area	Biology
More specific subject area	Skeletal muscle biology, cell physiology
Type of data	Image
How data was acquired	Western blotting
Data format	Raw
Experimental factors	Muscle satellite cells isolated from rats were cultured for 6 days to form myotubes and subjected to a whole cell protein extraction
Experimental features	The protein expression of adult slow MyHC isoforms was examined by Western blotting
Data source location	Fukuoka, Japan
Data accessibility	Data is supplied in this article

Value of the data

- The low but detectable amount of adult slow MyHC was confirmed in cultured myotubes differentiated from rat satellite cells for 6 days by Western blotting.
- The regulation of adult slow MyHC isoforms expression could be evaluated by Western blotting even though immature cultured myotubes.
- Undetectable level of adult fast MyHC isoforms appeared to be contained in myotubes in this experimental condition.

1. Data, experimental design, materials and methods

1.1. Sample preparation

Satellite cells were isolated from 4-week-old male Sprague–Dawley rats according to Allen et al. [2] with slight modification [3]. Briefly, muscles were excised, trimmed of fat and connective tissue, hand-minced with scissors, and digested for 1 h at 37 °C with 1.25 mg/ml protease type XIV (P5147; Sigma-Aldrich, USA). Cells were separated from muscle fiber fragments and tissue debris by differential centrifugation. In addition, to increase the purity of satellite cells in the cell mixture, cells were fractionated by Percoll density centrifugation according to Kastener et al. [4] with slight modification for a five-layered discontinuous density Percoll gradient (2 mL 27.5%–2 mL 35%–2 mL 40%–2 mL 55%–2 mL 90%). The fractionated cells (40–55% Percoll fraction) were plated at 2.0×10^4 cells/cm^2 on 6-well plates (4810-040; Iwaki brand Asahi Glass, Tokyo, Japan) coated with poly-l-lysine and fibronectin in alpha Modified Eagle's Minimum Essential Medium (α-MEM, Life Technologies, USA) containing 10% normal horse serum (HS, Invitrogen, USA), 1% antibiotic–antimycotic mixture (Life Technologies), and 0.5% gentamicin (Life Technologies). Cultures were placed in a humidified atmosphere of 5% CO_2 at 37 °C. Forty-eight hours after plating, satellite cells were differentiated in 2% HS in Opti-MEM (Life Technologies) for 6 days to form myotubes. These myotubes were lysed with 200 μl/well SDS solution containing 10% SDS, 40 mM dithiothreitol (DTT), 5 mM EDTA, and 0.1 M Tris–HCl buffer at pH 8.0, followed by addition of Protease Inhibitor Cocktail for use with mammalian cell and tissue extracts (1:100, Nacalai Tesque, Japan). These cell lysates were diluted by an equal volume of 2 × sample buffer (100 mM DTT, 4.0%w/v SDS, 0.16 M Tris–HCl (pH 6.8), 43% v/v glycerol, and 0.2% w/v bromophenol blue) and heated at 100 °C for 3 min. Animal experiments were performed according to the Guidelines for Animal Experiments of Kyushu University, and with the ethical approval of the Animal Care and Use Committee, Kyushu University (protocol 05-002-01)