PERGAMON

Neuromuscular Disorders 17 (2007) 569–574

www.elsevier.com/locate/nmd

Cytoplasmic γ-actin expression in diverse animal models of muscular dystrophy

Laurin M. Hanft [a], Daniel J. Bogan [b], Ulrike Mayer [c], Stephen J. Kaufman [d] Joe N. Kornegay [b], James M. Ervasti [a,*]

[a] *Department of Physiology, University of Wisconsin, Madison, WI, United States*
[b] *College of Veterinary Medicine and Dalton Cardiovascular Research Center, University of Missouri, Columbia, MO, United States*
[c] *Wellcome Trust Centre for Cell-Matrix Research, University of Manchester, Manchester, UK*
[d] *Department of Cell and Developmental Biology, University of Illinois, Urbana, IL, United States*

Received 19 December 2006; received in revised form 26 February 2007; accepted 5 March 2007

Abstract

We recently showed that cytoplasmic γ-actin (γ_{cyto}-actin) is dramatically elevated in striated muscle of dystrophin-deficient *mdx* mice. Here, we demonstrate that γ_{cyto}-actin is markedly increased in golden retriever muscular dystrophy (GRMD), which better recapitulates the dystrophinopathy phenotype in humans. γ_{cyto}-Actin was also elevated in muscle from α-sarcoglycan null mice, but not in several other dystrophic animal models, including mice deficient in β-sarcoglycan, α-dystrobrevin, laminin-2, or α7 integrin. Muscle from mice lacking dystrophin and utrophin also expressed elevated γ_{cyto}-actin, which was not restored to normal by transgenic overexpression of α7 integrin. However, γ_{cyto}-actin was further elevated in skeletal muscle from GRMD animals treated with the glucocorticoid prednisone at doses shown to improve the dystrophic phenotype and muscle function. These data suggest that elevated γ_{cyto}-actin is part of a compensatory cytoskeletal remodeling program that may partially stabilize dystrophic muscle in some cases where the dystrophin–glycoprotein complex is compromised.

Keywords: Duchenne muscular dystrophy; *mdx* Mouse; GRMD dog; γ-Actin; Sarcoglycan; α-Dystrobrevin; α7 Integrin; Utrophin

1. Introduction

Duchenne muscular dystrophy (DMD) is a severe, X-linked, progressive muscle disease affecting 1 in every 3500 male births. Mutations in the 2.5 million base pair DMD gene typically result in loss of the protein dystrophin [1]. Dystrophin functions as part of a larger oligomeric protein complex named the dystrophin–glycoprotein complex (DGC), which includes the dystroglycan subcomplex, the sarcoglycan/sarcospan subcomplex, dystrobrevins and syntrophins [2,3]. The DGC spans the sarcolemma and links the actin cytoskeleton with the extracellular matrix of myofibers [2,3].

We demonstrated that the DGC is required for strong mechanical coupling of costameric actin filaments to the sarcolemma and confirmed that sarcolemmal actin is exclusively comprised of the γ_{cyto}-actin isoform [4]. Transgenic expression of the dystrophin homolog utrophin restored the stable association of costameric actin with the sarcolemma [5]. Most recently, we demonstrated that γ_{cyto}-actin protein levels were elevated 10-fold in striated muscle from the dystrophin-deficient *mdx* mouse [6]. We hypothesized that elevated γ_{cyto}-actin levels may contribute to a compensatory remodeling of the dystrophin-

* Corresponding author. Address: Department of Biochemistry, Molecular Biology & Biophysics, University of Minnesota, 6-155 Jackson Hall, 321 Church Street SE, Minneapolis, MN 55455, United States. Tel.: +1 612 626 6517; fax: +1 612 625 2163.
E-mail address: jervasti@umn.edu (J.M. Ervasti).

0960-8966/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.nmd.2007.03.004

deficient costameric cytoskeleton [6]. While studies of *mdx* mice have greatly advanced our understanding of dystrophinopathies in humans, there are a number of important pathological differences between dystrophin-deficient humans and mice. Furthermore, mutations in genes encoding other DGC components or associated proteins have been implicated in clinically distinct forms of muscular dystrophy [2,3]. Finally, the complexity of the costameric protein network supports the hypothesis that additional proteins may form distinct mechanical linkages parallel to the DGC γ_{cyto}-actin axis. Therefore, it is of interest to determine whether the increased γ_{cyto}-actin measured in *mdx* muscle [6] manifests in other animal models of dystrophy or is unique to the *mdx* mouse.

Here, we report that γ_{cyto}-actin was also dramatically increased in the GRMD canine model of DMD and in a mouse model of limb-girdle muscular dystrophy 2D, but not in six additional mouse lines relevant to DGC function. Moreover, daily treatment of GRMD dogs with 2 mg/kg prednisone was previously shown to improve muscle function and overall phenotype [7] and is reported here to result in a further increase in γ_{cyto}-actin protein levels. We suggest that increased levels of γ_{cyto}-actin may participate in remodeling the costamere to partially reinforce the mechanically weakened dystrophin-deficient sarcolemma.

2. Materials and methods

2.1. Animals

C57BL/6J (6 or 16 weeks old), C57BL/10ScSn-DMD^{mdx}/J (16 weeks old), and C57BL/6J-Lama2^{dy} mice (6 weeks old) were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice deficient for α-sarcoglycan, β-sarcoglycan, α-dystrobrevin or α7 integrin were described previously [8–11] and were analyzed at 14–16 weeks of age. Transgenic mice overexpressing α7 integrin [12] were bred onto *mdx*, and *mdx*/utrophin −/− double knockout (*mdx*/utrn −/−) backgrounds and analyzed at 6 weeks of age. Muscle biopsies from control and GRMD dogs were described previously [7]. Where indicated, control and GRMD dogs were treated daily with 2 mg/kg from either 1 week to 6 months or 1 week to 2 months of age.

2.2. Antibodies

Affinity purified polyclonal antibodies and monoclonal antibodies to γ_{cyto}-actin were previously characterized [6]. Antibodies to sarcomeric α-actin were from Sigma (St. Louis, MO). Peroxidase-labeled secondary antibodies against rabbit and mouse immunoglobulins were from Chemicon International, Inc. (Temecula, CA) and Roche (Indianapolis, IN), respectively.

2.3. Muscle extracts and DNaseI chromatography

Frozen skeletal muscle was pulverized in a liquid nitrogen-cooled mortar and pestle, extracted in Tris-buffered saline and enriched for soluble monomeric actin by DNaseI affinity chromatography as described [6].

2.4. SDS–PAGE and Western blot analysis

SDS–polyacrylamide gel electrophoresis, transfer to nitrocellulose and Western blotting were performed as described [6].

2.5. Quantitative Western blot analysis

γ_{cyto}-Actin immunoreactivity was detected from Western blots by autoradiography using ^{125}I-labeled goat anti-mouse IgG as secondary (Perkin-Elmer, Boston, MA) and quantified densitometrically as previously described [6]. Statistical comparisons between groups were performed by ANOVA followed by a non-paired Tukey post hoc test to determine significance and data are reported as the means ± SD.

3. Results and discussion

To determine whether γ_{cyto}-actin is dramatically elevated in other dystrophin-deficient animals besides the *mdx* mouse, DNaseI-enriched muscle extracts from control and GRMD dogs were compared for γ_{cyto}-actin

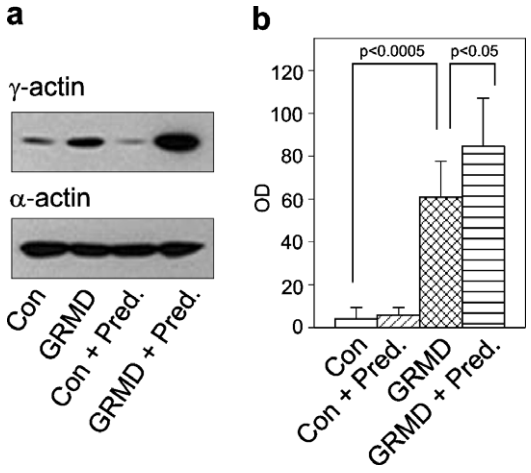

Fig. 1. γ_{cyto}-Actin levels in dystrophin-deficient GRMD skeletal muscle. (a) Western blots loaded with DNaseI-enriched muscle extracts from untreated control (Con), GRMD, and prednisone-treated control or GRMD animals were stained for γ_{cyto}-actin and α-actin. (b) Quantitation of γ-actin immunoreactivity (OD, arbitrary units) in DNaseI eluates from muscle in control ($n = 4$), GRMD ($n = 7$), prednisone-treated control ($n = 4$), and prednisone-treated GRMD animals ($n = 6$). γ_{cyto}-Actin was significantly elevated in GRMD muscle compared to control ($p < 0.0005$) and in muscle from prednisone-treated GRMD compared to untreated GRMD animals ($p < 0.05$).