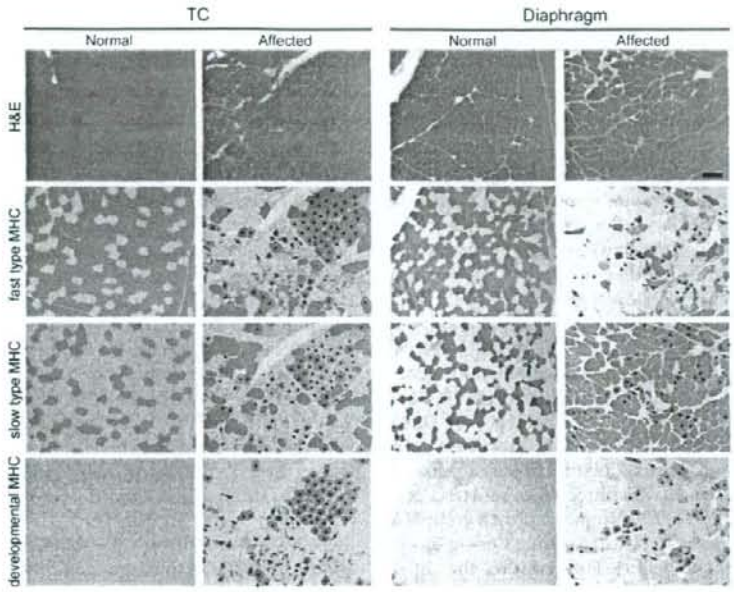

Figure 1
Representative images of histology (H&E) and expression of fast type, slow type, or developmental myosin heavy chain (MHC) in tibialis cranialis (TC) muscle and diaphragm of a normal (10 months old) or a $CXMD_J$ dog (11 months old). Identical parts of serial cross-sections are shown in longitudinal panels. In panels of affected muscles, dots show the fibers expressing developmental MHC. Bar: 200 μm.

alteration of MHC expression and regeneration of muscle fibers would be different between TC muscle and the diaphragm.

Time courses of histology and MHC expression

To investigate how MHC expression alters together with growth of $CXMD_J$, we examined MHC expression in TC muscles and diaphragms of a normal or an affected littermate at various ages from neonatal to adult stages (1 month to 1 year old) in relation to histopathological features. Affected TC muscles showed mild lesions at 1 and 2 months old, but severe degenerative lesions were evident at over 4 months old (Fig. 3). Expression of fast or slow type MHC did not alter much with aging, and developmental MHC was expressed continuously (Fig. 4). In contrast, degenerative lesions were severe in the affected diaphragm at all ages examined (from 1 month old onward), and endomysial fibrosis was dominantly present over 6 months old (Fig. 3). Fast MHC fiber number decreased markedly, while the number of slow MHC fibers increased significantly in affected diaphragms after 6 months old (Fig. 5). In addition, expression of developmental MHC decreased at 6 months and 1 year old. These observations indicated that MHC expression is altered greatly in the affected diaphragms after 6 months old, unlike TC muscles.

For quantitative evaluation of MHC expression in individual myofibers, we counted three types of MHC-expressing fibers among non-regenerating or regenerating populations within an area in the TC muscle or diaphragm of a normal or an affected littermate (Fig. 6). As normal muscles still expressed developmental MHC at 1 month old (Fig. 4 and 5), we performed the examinations at both adolescent (2 and 4 months old) and adult stages (10 or 11 months old). In normal dogs, the number of fast MHC fibers in TC muscle was three times greater than that of slow MHC fibers throughout aging, while the proportions in the diaphragms remained constant and equivalent between the two types (Fig. 6A). In non-regenerating fibers, the proportions of fiber types were not constant in affected TC muscles at the ages examined, but the majority of these fibers consisted of slow MHC fibers in the affected diaphragms (Fig. 6B). These observations indicated that slow fibers were already predominant in non-regenerating populations of CXMDJ diaphragms at younger ages. In

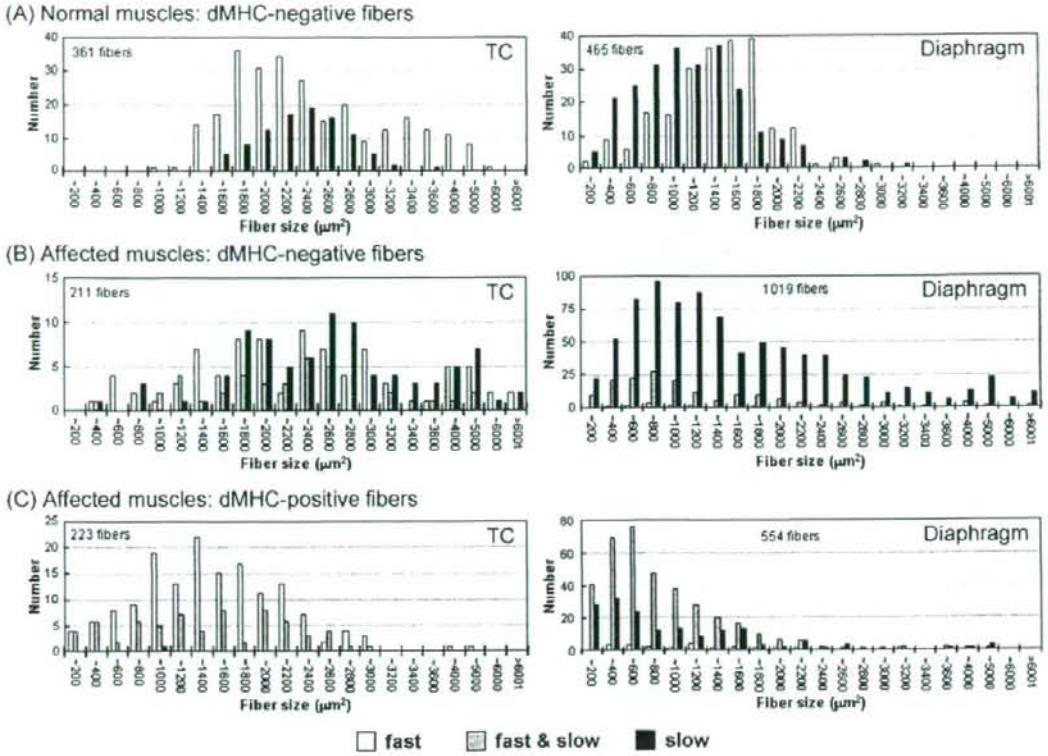

Figure 2

The size distribution of myofibers expressing fast and/or slow type MHCs in skeletal muscles of a normal (10 months old) or a $CXMD_J$ dog (11 months old). On the basis of expression of fast and slow type MHCs, all fibers within an area of TC muscle or diaphragm of a normal (A) or an affected dog (B, C) were classified into three types of MHC-positive fiber. Furthermore, fast (white), hybrid (gray), or slow MHC myofibers (black) were analyzed among populations of muscle fibers with non-expression of developmental MHC (A, B) or with expression of developmental MHC (C) in terms of fiber numbers (see Table 1) and fiber sizes (A-C). Note that larger sizes of slow MHC fibers were noticeable in populations of muscle fibers expressing fast and/or slow MHC(s) but not developmental MHC of affected muscles (B).

regenerating fibers, in contrast to the observation that fast MHC fibers consistently accounted for the majority of fibers in affected TC muscles, the affected diaphragms were mainly composed of hybrid and slow MHC fibers and the proportion increased gradually with age (Fig. 6C). These observations indicated that MHC expression in regenerating fibers was also different between affected TC muscle and diaphragm after 4 months old, although it was relatively similar in the two at 2 months old.

Temporal changes of MHC isoforms

To examine how progressive degeneration alters the composition of fiber types in affected skeletal muscles, we detected myosin isoforms in TC muscles and diaphragms of $CXMD_J$ at various ages by electrophoretic gel separation (Fig. 7). Four MHC isoforms (I, IIA, IIX, and embryonic), which migrated on electrophoresis as IIA-embryonic-IIX-I from slowest to fastest [11,12], were detected in canine skeletal muscles (Fig. 7A). In affected TC muscles, type I, IIA, and embryonic isoforms were consistently detected at similar levels, but the level of type IIX MHC was lower than those in normal TC muscles after 2 months old. In contrast, type IIA MHC level decreased gradually in affected diaphragms with growth, and type I accounted for the majority of MHC components in animals over 6 months old. In addition, the embryonic isoform

Table 1: The numbers of myofibers co-expressing fast type, slow type, and/or developmental MHCs in skeletal muscles of a normal (10 months old) or a CXMD$_J$ dog (11 months old).

	TC			Diaphragm		
	Normal	Affected		Normal	Affected	
Developmental	-	-	+	-	-	+
Fast	265 (73%)	85 (40%)	155 (70%)	222 (48%)	12 (1%)	20 (3.6%)
Fast & slow	0 (0%)	38 (18%)	67 (30%)	0 (0%)	160 (16%)	370 (66.8%)
Slow	96 (27%)	88 (42%)	1 (0%)	243 (52%)	847 (83%)	164 (29.6%)
Total	361 (100%)	211 (49%)	223 (51%)	465 (100%)	1019 (65%)	554 (35%)

The numbers of fibers analyzed were results from a normal or an affected dog. MHC expression between two groups (normal, dMHC (-) *vs* affected, dMHC (-); affected, dMHC (-) *vs* affected, dMHC (+)), or between muscles (TC muscle *vs* diaphragm) was analyzed by Yates's chi-square test. Significant differences ($p < 0.05$) were detected in all tests.

decreased in affected diaphragms after 6 months old. These results were consistent with those of immunohistochemical analyses (Figs. 4 and 5). These observations suggested that type IIX and IIA fast fibers may be preferentially affected in TC muscle and diaphragm of CXMD$_J$, respectively. Furthermore, these observations suggested that muscle regeneration may deteriorate from relatively younger age in the affected diaphragm, unlike TC muscle.

Discussion

To investigate the alterations in fiber types in skeletal muscles of a canine DMD model, we examined MHC expression in the TC muscle and diaphragm of CXMD$_J$ at various ages. Our results indicated that the influences of dys-

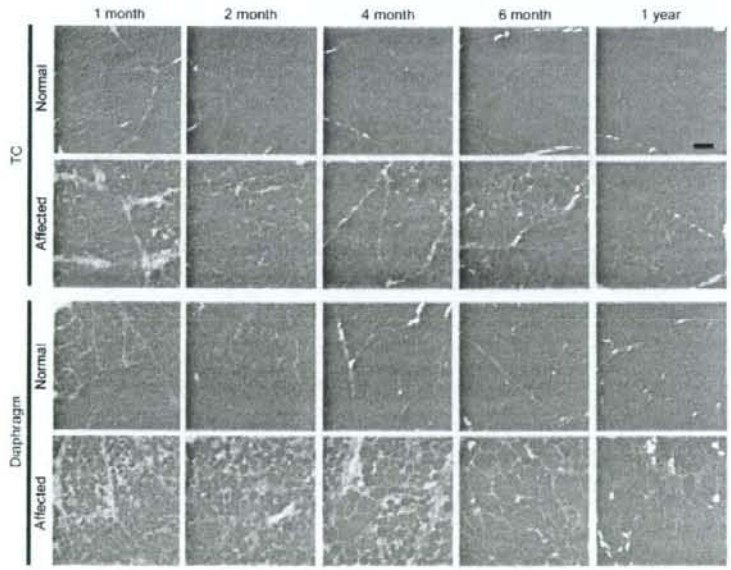

Figure 3
Representative histological findings in TC muscles and diaphragms of a normal or a CXMD$_J$ dog at 1, 2, 4, 6 months, and 1 year old. Note that severe degenerative lesions were observed from early ages in affected diaphragms, as compared with affected TC muscles. Bar: 200 μm.

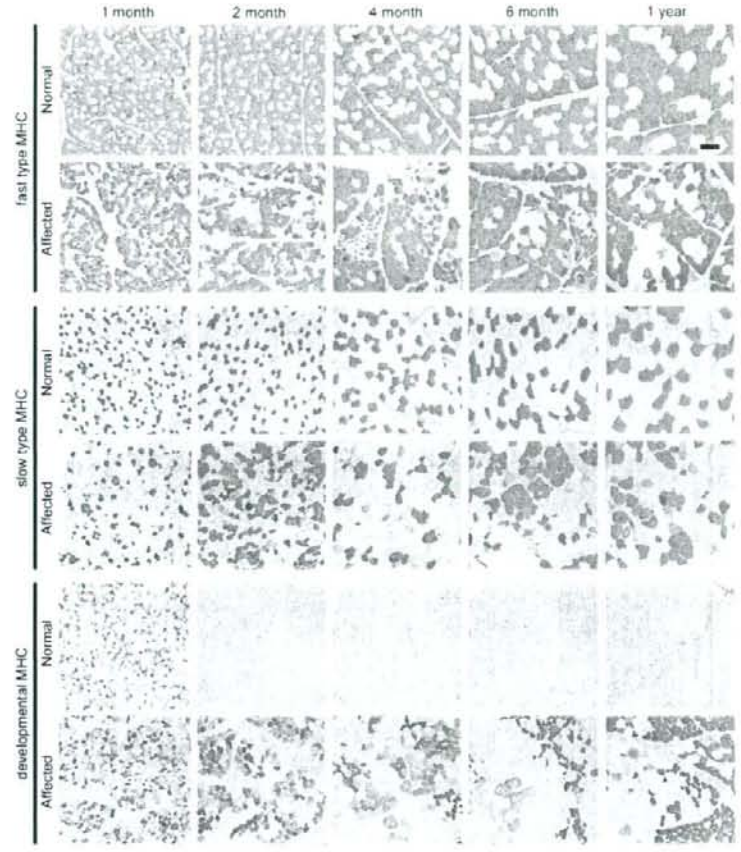

Figure 4
Expression of fast type, slow type, and developmental MHCs in TC muscles of a normal or a CXMD$_J$ dog at 1, 2, 4, 6 months, and 1 year old. Note that there were no notable differences between expression levels of fast and slow type MHCs in normal and affected TC muscles. Bar: 200 μm.

trophin deficiency on fiber type composition were significantly different between TC muscle and diaphragm.

To analyze MHC expression in details, we compared fiber type composition and fiber size distribution of MHC-expressing fibers between a normal dog (10 months old) and an affected dog (11 months old). In normal and affected dogs, body weight rapidly increased to approximately 9 kg at 4 months old, and then slightly increased to approximately 14 and 11 kg at 12 months old, respectively [5]. As body weight reflects muscle weight, muscle mass and fiber size would not extremely change in 1 month after 4 months old, especially in normal dogs. In fact, in TC muscles or diaphragms of normal dogs, there were no significant differences among compositions of fiber types and MHC isoforms after 4 months old (Fig 6 and 7). In addition, we examined normal dogs at 11, 12 and 14 months old, and affected dogs at 10, 12, 13 and 15 months old. Normal muscles of adult dogs showed similar expression of fast type, slow type, or developmental MHC at all adult ages, and affected muscles also showed similar MHC expression at examined ages (data not shown). These observations implied that there would be no significant difference in MHC expression between at 10 and 11 months old, in both of normal and affected dogs.

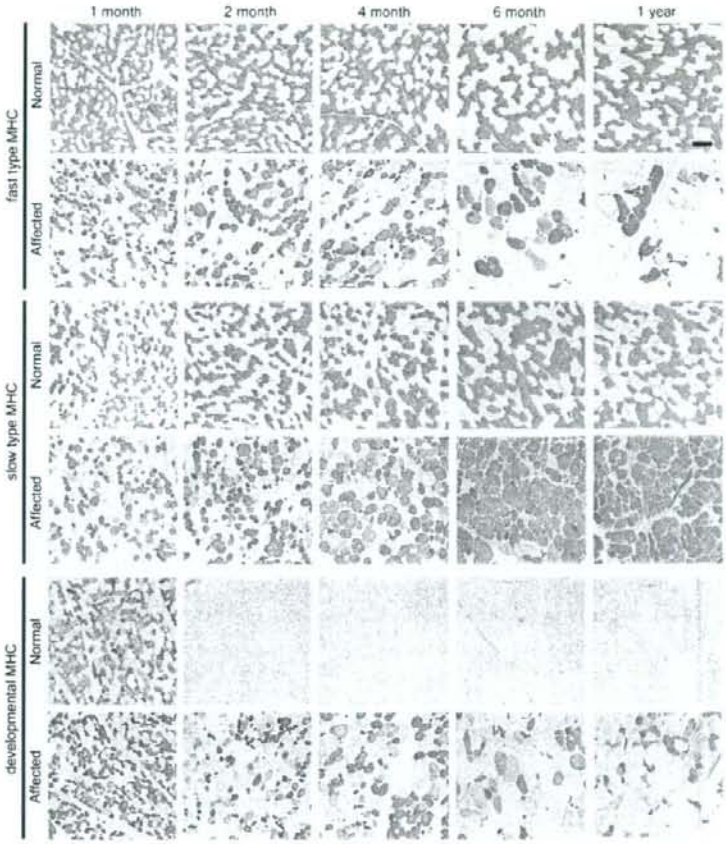

Figure 5
Expression of fast type, slow type, and developmental MHCs in diaphragms of a normal or a CXMD$_J$ dog at 1, 2, 4, 6 months, and 1 year old. Note that slow MHC fibers were increased markedly in the affected diaphragms after 6 months old, while fast MHC fibers were decreased. Bar: 200 μm.

Common features between TC muscle and diaphragm of CXMD$_J$

TC muscle and diaphragm of CXMD$_J$ shared the features that slow MHC fibers increased and enlarged selectively in non-regenerating populations, while fast type IIX or IIA MHC isoform decreased. Similar observations have been reported in skeletal muscles of the *mdx* mouse [13], GRMD [14], and human DMD [12,21]. In general, increasing and enlarging of slow fibers may be a consequence of adaptive responses by metabolic enzyme systems and energy consumption, because slow fibers have lower capacity for power output and consume less energy than fast fibers [22]. Our results also supported the hypothesis that slow fibers would be more adaptable to dystrophic stress than fast fibers, to compensate for the reduced abilities of muscle function.

Two mechanisms were considered to explain the selective increase in slow fibers during progressive muscle degeneration. One possibility is that slow fibers may be more resistant to dystrophic stress than fast fibers, leading to selective survival of slow fibers. This was supported by the observation that slower muscle fibers contained significantly more utrophin, a homolog of dystrophin, in comparison to faster counterparts [23,24]. Another is transition of MHC isoforms, where type IIA or IIX MHC

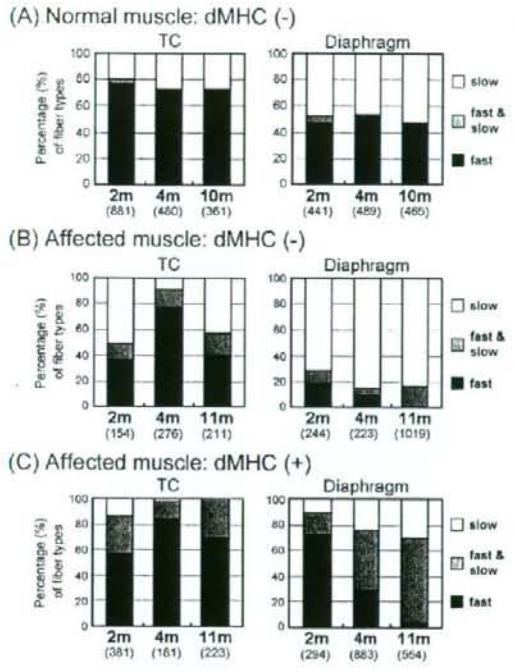

Figure 6

Proportions of fiber types in skeletal muscles of a normal or a $CXMD_J$ dog at various ages. The numbers of fast (black), hybrid (gray), and slow MHC myofibers (white) among populations of myofibers without developmental MHC (A, B) and with developmental MHC (C) were counted in TC muscle and diaphragm of a normal (A) or an affected dog (B, C) at adolescent (2 or 4 months old) or adult stages (10 or 11 months old). The numbers under the ages show total fibers examined. MHC expression between two groups (normal, dMHC (-) *vs* affected, dMHC (-); affected, dMHC (-) *vs* affected, dMHC (+)), between muscles (TC muscle *vs* diaphragm), or among ages (2, 4, and 10 or 11 months) was analyzed by Yates's chi-square test. Significant differences ($p < 0.05$) were detected in all tests, except for no significant differences between 4 and 10 months old in normal TC muscles or diaphragms. Note that slow MHC fibers were consistently larger than other fibers, in populations of muscle fibers without developmental MHC of affected diaphragms. In populations of muscle fibers co-expressing developmental MHC and other MHC isoform(s), slow MHC and hybrid fibers were increased markedly in the affected diaphragm at 4 and 11 months old, unlike TC muscles.

isoforms could be transited to type I, as seen in hypertrophy and exercise [25]. MHC I, IIa, IIx, and IIb gene expression are known to be regulated by the calcineurin pathway [26,27]. Dystrophin deficiency may accelerate MHC transition to slower types *via* calcineurin/NFAT signaling in skeletal muscles of $CXMD_J$, because calcineurin and activated NFATc1 protein content were higher in muscles from *mdx* than wild-type mice [28]. However, it remains possible that both mechanisms may be active at the same time, because the calcineurin/NFAT cascade can regulate not only the MHC promoters but also the utrophin A promoter [24,29,30].

Differences between TC muscle and diaphragm of $CXMD_J$

The $CXMD_J$ diaphragm developed severe degenerative lesions from earlier stages than TC muscle, which corresponded to previous reports [3,5,31]. In addition, dystrophic changes in the $CXMD_J$ diaphragm not only markedly altered the expression of fast and slow type MHCs but also decreased the amount of the developmental (embryonic and/or neonatal) MHC with growth, unlike affected TC muscle. Especially, fast MHC fibers disappeared and slow MHC fibers enlarged in the adult $CXMD_J$ diaphragm. The greater cross-sectional area of slow fibers in affected diaphragms might be due to hypertrophy in compensation for loss of fast fibers, relating to plasticity of muscle fibers, as mentioned above. The diaphragm keeps continuous contraction of muscle fibers without resting, while limb skeletal muscle regularly rests its movement. Therefore, replacement with slow fibers may be particularly enhanced in the diaphragm rather than TC muscle, depending on pathological severity and contractile activity of skeletal muscles.

Fiber type determination and fiber type-specific gene expression are regulated by multiple signaling pathways and transcription factors. As partially described above, a key mediator, calcineurin, plays an important role in acquisition of fiber phenotype [29,30] and may induce not only transition of MHC isoforms from faster to slower types but also transformation of myofiber phenotypes in mouse or rat muscles [26,27,32]. In addition, calcineurin signaling activity was greater in the diaphragm than in the tibialis anterior muscle of the *mdx* mouse [28]. Therefore, replacement with slow fibers may be up-regulated to a greater extent in the diaphragm than in the TC muscle of $CXMD_J$.

We also showed age-related changes of MHC expression in affected diaphragms after 6 months old, in contrast to TC muscles (Fig 4, 5 and 7). In addition, fiber type compositions in non-regenerating or regenerating fibers were also different between the TC muscle and the diaphragm, depending on age. In non-regenerating fibers of affected TC muscles, fast MHC fibers at 4 months old was higher than those at 2 and 11 months old (Fig. 6B). It might be partially involved in pathological changes that degenerative lesions appeared obviously in affected TC muscles after 4 months old, as described previously [3,5,31]. In

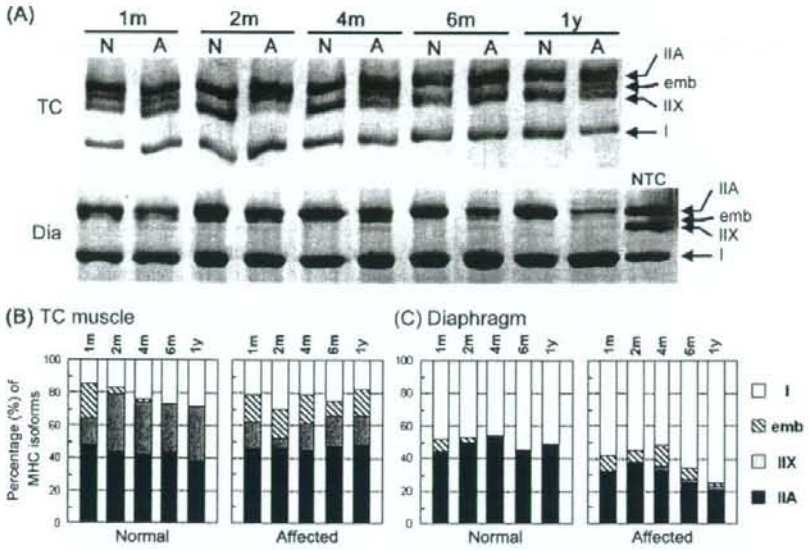

Figure 7

MHC isoforms in skeletal muscles of normal and CXMD$_J$ dogs. (A) Electrophoretic separation of MHC isoforms in TC muscle and diaphragm. Myosin was extracted from muscles at various ages (1, 2, 4, 6 months, and 1 year old), and aliquots of 0.4 μg of protein were separated on 8% SDS-polyacrylamide gels containing 30% glycerol. Four MHC isoforms (I, IIX, IIA, and embryonic) were detected. NTC: normal TC muscle at 1 year old. Note that MHC type I increased in the affected diaphragm after 6 months old. (B) Quantitative analysis of MHC isoforms. MHC expression between two groups (normal *vs* affected) or among ages (1, 2, 4, 6 months and 1 year) was analyzed by Yates's chi-square test. Significant differences ($p < 0.05$) were detected between normal and affected groups in TC muscles after 2 months old or in diaphragms after 4 months old, and between 1 and 2 months old in normal TC muscles.

regenerating fibers of the CXMD$_J$ diaphragm, the proportion of myofibers expressing slow type MHC increased markedly after 4 months old (Fig. 6C). These results suggested that MHC expression in TC muscle and the diaphragm of CXMD$_J$ would be influenced by different mechanisms after 4 months old. These age-dependent MHC expression might be related to body growth, particularly increasing of muscle mass. One possibility is participation of insulin-like growth factor (IGF)-1, which is important for postnatal growth of skeletal muscles [33] and can activate multiple Ca^{2+}-dependent signaling pathways, including the calcineurin/NFAT pathway [30]. When growth rate of body weight decreases after 4 months old [5], signaling activity of IGF-1 might reduce and MHC expression might be regulated predominantly by alternative signaling pathways.

Comparison among mdx, CXMD$_J$, and DMD diaphragms

MHC expression in normal skeletal muscle has been well studied in mice [15,34], dogs [11], and humans [35]. In normal dogs, the proportions of fiber types in TC muscle were relatively similar to those in the representative tibialis anterior muscles of mice and humans. In the diaphragm, however, the proportion of fiber types differed markedly among these species. The murine diaphragm is composed mainly of fast type IIA and IIX isoforms [15,34], but the canine diaphragm consists of equal populations of slow type MHC I and fast type MHC IIA [11], as also shown in our study. In normal human diaphragm, the distribution of myosin isoforms has been estimated that types I, IIA, and IIX account for approximately 45%, 40%, and 15%, respectively [35]. Thus, the proportions of MHC isoforms in the diaphragm of healthy dogs are much closer to those of humans than those of mice.

Some groups have studied expression profiles of MHC isoforms in the diaphragm of the *mdx* mouse. The *mdx* diaphragm shows increases in MHC type I fibers and elimination of type IIX population at 2 years old, but not at young ages (3 to 6 months old) [13,15,34]. In contrast to the *mdx* diaphragm, that in CXMD$_J$ exhibited drastic changes even in younger animals (6 months old). On the

other hand, there is no direct information available regarding the changes in fiber type composition in the diaphragm in human DMD. In addition, there is an important difference of MHC expression even in limb skeletal muscles between large mammals (including dogs and humans) and mammals with smaller body mass, especially rodents. The former do not express the fastest MHC IIB isoform in limb muscles [10,11,36], while it is abundantly expressed in the latter [34]. Therefore, changes/adaptations in skeletal muscles of dogs with muscular dystrophy are likely to be more relevant to human DMD, than that in the *mdx* mouse. As it is difficult to examine the diaphragms of DMD patients, it would be important to investigate the differences between murine and canine models for understanding the mechanisms of respiratory failure in human DMD.

Conclusion

Based on fiber type classification using MHC expression, we demonstrated the predominant replacement with slow fibers and reduced muscle regeneration with progression of muscular dystrophy in the diaphragm of a canine DMD model, but these phenomena were much less strict in affected TC muscle. In addition, the expression profiles of MHC isoforms in the $CXMD_J$ diaphragm were evidently different from those of the *mdx* mouse. Our results indicated that dystrophic dog is a more appropriate model than a murine one for human DMD, and would be useful for investigation of the mechanisms of respiratory failure in DMD, as well as pathological and molecular biological backgrounds, and therapeutic effects in clinical trials.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

KY designed the study, carried out the pathological and immunohistological examinations, and drafted the manuscript. AN participated in interpretation of data, and helped to draft the manuscript. TH participated in coordination of the study. ST participated in the design, planning, and coordination of the study, and helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We are grateful to Dr. Madoka Yoshimura, Dr. Nobuyuki Urasawa, Dr. Naoko Yugeta, Ms. Ryoko Nakagawa, and Dr. Masayuki Tomohiro for technical assistance. We also thank Mr. Hideki Kita and Mr. Shinichi Ichikawa for care and management of experimental animals. This work was supported by Grants-in-Aid for Center of Excellence (COE), Research on Nervous and Mental Disorders (13B-1, 16B-2, 17A-10, 19A-7), Health Science Research Grants for Research on Psychiatry and Neurological Disease and Mental Health (H12-kokoro-025, H15-kokoro-021, H18-kokoro-019), and the Human Genome and Gene Therapy (H13-genome-001, H16-genome-003) from the Ministry of Health, Labor, and Welfare of Japan, and Grants-in-Aid for Scientific Research to KY and High-Tech Research Center Project for Private Universities (matching fund subsidy, 2004-2008) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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Pre-publication history

The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1471-2474/9/1/prepub

Acta Myologica • 2008; XXVII; p. 14-18

Myostatin inhibition by a follistatin-derived peptide ameliorates the pathophysiology of muscular dystrophy model mice

K. TSUCHIDA

Division for Therapies against Intractable Diseases, Institute for Comprehensive Medical Science (ICMS), Fujita Health University, Toyoake, Aichi, Japan

Gene-targeted therapies, such as adeno-associated viral vector (AAV)-mediated gene therapy and cell-mediated therapy using myogenic stem cells, are hopeful molecular strategies for muscular dystrophy. In addition, drug therapies based on the pathophysiology of muscular dystrophy patients are desirable. Multidisciplinary approaches to drug design would offer promising therapeutic strategies. Myostatin, a member of the transforming growth factor-β superfamily, is predominantly produced by skeletal muscle and negatively regulates the growth and differentiation of cells of the skeletal muscle lineage. Myostatin inhibition would increase the skeletal muscle mass and prevent muscle degeneration, regardless of the type of muscular dystrophy. Myostatin inhibitors include myostatin antibodies, myostatin propeptide, follistatin and follistatin-related protein. Although follistatin possesses potent myostatin-inhibiting activity, it works as an efficient inhibitor of activins. Unlike myostatin, activins regulate the growth and differentiation of nearly all cell types, including cells of the gonads, pituitary gland and skeletal muscle. We have developed a myostatin-specific inhibitor derived from follistatin, designated FS I-I. Transgenic mice expressing this myostatin-inhibiting peptide under the control of a skeletal muscle-specific promoter showed increased skeletal muscle mass and strength. *mdx* mice were crossed with FS I-I transgenic mice and any improvement of the pathological signs was investigated. The resulting *mdx*/FS I-I mice exhibited increased skeletal muscle mass and reduced cell infiltration in muscles. Muscle strength was also recovered in *mdx*/FS I-I mice. Our data indicate that myostatin inhibition by this follistatin-derived peptide has therapeutic potential for muscular dystrophy.

Key words: Myostatin, follistatin, muscular dystrophy

Actions of Myostatin

Skeletal myogenesis is under tight regulation by growth factor signaling. Myostatin is an endogenous negative regulator of muscle growth and plays a major role in determining skeletal muscle mass. Myostatin, also known as growth and differentiation factor-8 (GDF8), belongs to the transforming growth factor (TGF)-β superfamily (1, 2). Similar to other TGF-β superfamily members, myostatin is synthesized as a precursor protein that is biologically inactive. Production of mature myostatin occurs through dimerization of the precursor and subsequent proteolytic processing. Cleavage by furin-like protease is responsible of separating the N-terminal propeptide from the C-terminal mature myostatin, while cleavage of the latent propeptide by the bone morphogenetic protein-1/tolloid (BMP1/TLD) family of metalloproteinases is responsible for activation of latent myostatin (3). The C-terminal dimeric 26-kDa protein acts as mature myostatin. Mice with targeted deletion of the myostatin gene show dramatic and widespread increases in skeletal muscle mass (2). Both muscle fiber hypertrophy and muscle cell hyperplasia are observed.

Myostatin signals through two types of transmembrane serine/threonine kinase receptors, namely activin type II receptors (ACVR2B and ACVR2A) and activin receptor-like kinases 4 and 5 (ALK4 and 5). Its intracellular signaling pathway is similar to those of activin and TGF-β, and mediated by the Smad proteins Smad2 and Smad3 (1, 2, 4). Myostatin negatively regulates G1-to-S progression in the cell cycle and maintains the quiescent status of satellite cells (5). As a result, increased numbers of satellite cells are present in myostatin-deficient mice (5). Involvement of the MAP kinase pathway as well as the Smad pathway is a characteristic of the myostatin-regulated skeletal muscle differentiation program (6). However, the precise mechanism of action and the skeletal-muscle specific signaling of myostatin have not yet been fully elucidated.

Address for correspondence: Kunihiro Tsuchida, Institute for Comprehensive Medical Science (ICMS), Fujita Health University, Toyoake, Aichi 470-1192, Japan. E-mail: tsuchida@fujita-hu.ac.jp

Myostatin Inhibition as a Therapeutic Strategy for Muscular Dystrophy

Interestingly, inhibition of myostatin activity is capable of increasing muscle mass and strength in the postnatal period and even in adults. These observations suggest that targeting of myostatin would be a suitable therapy for degenerative muscle diseases, such as muscular dystrophy and cachexia, and may be able to prevent muscle wasting due to aging (1, 2, 7). In fact, antibody-mediated myostatin blockade in *mdx* mice, a model for Duchenne muscular dystrophy, was found to ameliorate the pathophysiology and muscle weakness (8). Myostatin propeptide-mediated amelioration of the symptoms in *mdx* mice, limb-girdle muscular dystrophy (LGMD) 1C model mice with caveolin-3 gene mutations and LGMD2A model mice with calpain 3 gene mutations has also been reported (9-11). However, elimination of myostatin did not recover the pathology in laminin-α2-deficient model mice and rather increased their mortality (12). Thus, the effectiveness of myostatin inhibition depends on the disease state (Table 1). In addition to myostatin propeptide and myostatin antibodies, follistatin and follistatin domain-containing proteins can bind to myostatin *in vivo* and act as effective myostatin inhibitors (1, 13, 14). Small chemical compounds that block the kinase activity of myostatin type I receptor would also serve as myostatin inhibitors (13).

Development of Myostatin Inhibitors for Therapies against Muscular Dystrophy

Phage display technology and antibody engineering have been used to develop myostatin-blocking antibodies. The biosafety and effectiveness of humanized myostatin antibodies, designated MYO-029, are being evaluated in phase I/II studies in the United States in 108 patients suffering from muscular dystrophy (3).

Multiple myostatin-binding proteins, such as myostatin propeptide, follistatin and follistatin-related protein, have been characterized. After cleavage of myostatin precursors, myostatin propeptide associates with mature myostatin in sera (14). Proteolytic cleavage of the propeptide at aspartate-76 by the BMP-1/TLD family of metalloproteinases is an important step for activa-

Table 1. Muscular dystrophies and myostatin inhibition.

Disease	Mode of inheritance	Gene locus	Gene products	Myostatin blockage	Ref [Method of myostatin inhibition]
Duchenne	XR	Xp21	Dystrophin	Effective in *mdx* mouse	Bogdanovich et al., (8) [1] Wagner et al., (21) [2] Bogdanovich et al., (9) [3] Nakatani et al., (17) [4]
LGMD1C (CAV3)	AD	3p25	Caveolin-3	Effective in model mouse	Ohsawa et al., (10) [5]
LGMD2A (CAPN3)	AR	15q15	Calpain-3	Gene therapy is effective	Bartoli et al., (11) [6]
LGMD2D (SGCA)	AR	17q12-21	α-sarcoglycan	Gene therapy is not effective	Bartoli et al., (11) [6]
LGMD2F (SGCD)	AR	5q33-34	δ-sarcoglycan	Early therapy is effective Treat early	Parsons et al., (22) [1, 2]
MDC1A (LAMA2)	AR	6q22	Laminin α-2	Not effective in *dy* mouse Severe fat loss	Li et al., (12) [2]

The effects of myostatin blockade on various types of muscular dystrophy are summarized. Myostatin inhibition is applicable as a therapy for multiple types of muscular dystrophy. Transgenic approaches, systemic injection and gene therapy have been tried. Myostatin blockade by myostatin antibodies, modified myostatin propeptide or follistatin-derived peptides is effective for ameliorating the pathophysiology in *mdx* mice. Myostatin inhibition is also effective for ameliorating several types of limb-girdle-type muscular dystrophy caused by mutations of caveolin-3 or calpain-3. Effective therapy would be possible by early treatment. It is noteworthy that elimination of myostatin does not improve the phenotypes of laminin-α2-deficient model mice. Method of myostatin inhibition is shown as brackets. [1], myostatin antibody treatment; [2], crossing with myostatin K/O mice; [3], myostatin propeptide treatment; [4], crossing with mutated follistatin Tg mice; [5], crossing with myostatin propeptide Tg mice; [6], AAV-mediated mutated myostatin propeptide expression. References are shown with parentheses.

tion of the mature disulfide-bonded C-terminal myostatin dimer (2, 3). Mutation of the myostatin propeptide at the BMP-1/TLD cleavage site by replacing aspartate-76 with alanine (D76A) produces a better myostatin inhibitor than the wild-type propeptide *in vitro* and *in vivo* (9, 11).

Although the activin type IIB receptor, ACVR2B, is characterized as a receptor for activins and nodal, it is the primary ligand-binding myostatin receptor that transmits myostatin signaling. A soluble form of ACVR2B has potent myostatin-inhibitory activity and causes dramatic increases in muscle mass (15). Only 2 weeks are required for the soluble form of ACVR2B to increase the muscle mass in mice by up to 60% (15). Since the soluble form of ACVR2B even augments muscle mass in myostatin-knockout mice, it has been suggested that it also inhibits other ligands including activins and GDF11 that regulate skeletal muscle growth in addition to myostatin (15).

Myostatin Inhibitor Derived from Follistatin

Follistatin was originally identified as a single-chain polypeptide with a weak inhibitory activity toward follicle-stimulating hormone secretion by anterior pituitary cells. Later, follistatin was found to be an activin-binding protein (1). Gene knockout analyses revealed that follistatin gene ablation causes multiple effects, including skeletal and skin abnormalities, suggesting that follistatin may have additional functions other than activin inhibition (1). Follistatin and follistatin-related gene, FLRG, were shown to bind to myostatin and inhibit its activity (1, 2, 15, 16). Similar to myostatin, activins belong to the TGF-β superfamily and have pleiotrophic effects on numerous tissues. Since activins have a variety of functions in tissues other than skeletal muscles and their inhibition by follistatin is very efficient, follistatin has multiple effects on not only skeletal muscles but also other tissues. In fact, transgenic expression of the follistatin gene has profound effects on reproductive performance and fertility (1).

Recently, we developed a myostatin inhibitor derived from follistatin, designated FS I-I, and characterized its effects on muscle mass and strength in *mdx* mice (17). Since myostatin blockade is one of the most promising therapies for muscular dystrophy, the results of our study should provide an additional rational therapeutic strategy for intractable muscular diseases, including muscular dystrophy (17).

Follistatin is composed of an N-terminal domain and three cysteine-rich follistatin domains (FS I, FS II and FS III) (1). Recent crystallographic analyses have revealed that the minimal activin-inhibiting fragment of follistatin is comprised of the FS I and FS II domains, and that the individual FS domains may have different activities (18, 19). We created a follistatin mutant containing two FS I domains, and characterized its binding activities toward myostatin and activin A. Interestingly, FS I-I retained its myostatin binding, but showed significantly weaker activin-binding activity. The dissociation constants of follistatin for activin and myostatin are 1.72 and 12.3 pM, respectively. In contrast, the dissociation constants of FS I-I for activin and myostatin are 64.3 nM and 46.8 pM, respectively. FS I-I was capable of inhibiting the actions of myostatin in multiple assays, but hardly affected the activin activity (17). Transgenic mice expressing FS I-I under the control of a skeletal muscle-specific promoter showed increased skeletal muscle mass, especially in the pectoralis major, triceps brachii, gluteus and quadriceps femoris muscles. Muscle strength was also increased. Hyperplasia and hypertrophy were both observed. FS I-I transgenic mice did not show any behavioral abnormalities and reproduced normally. We crossed FS I-I transgenic mice with *mdx* mice, a model for Duchenne muscular dystrophy. Notably, the skeletal muscles in the resulting *mdx*/FS I-I mice were enlarged and showed reduced cell infiltration (17). The numbers of infiltrated macrophages in skeletal muscles were dramatically decreased in *mdx*/FS I-I mice compared with *mdx* mice (17). Muscle strength was also recovered in *mdx*/FS I-I mice. These results indicate that myostatin blockade by FS I-I has therapeutic potential for muscular dystrophy and should provide a rational therapeutic strategy for intractable muscular diseases. The possibility that injections of this myostatin inhibitor derived from follistatin may affect the pathophysiology of muscular dystrophy model mice or human patients remains to be determined.

Conclusions

The ability to control the actions of myostatin has great potential for a number of research fields and offers medical applications. Myostatin activity determines the skeletal muscle mass. Myostatin blockade is effective for increasing muscle mass, even in adults (1, 2). Thus, myostatin is considered to be one of the rational drug targets for muscle-wasting diseases, such as muscular dystrophy. There are multiple strategies for inhibiting myostatin activity. Myostatin inhibitors, such as monoclonal myostatin antibodies, myostatin propeptide and follistatin, could be promising lead compounds in drug development for muscular dystrophy and related disorders (1, 2, 17).

There are various types of muscular dystrophy, including Duchenne/Becker muscular dystrophies, congenital muscular dystrophies and limb-girdle muscular dystrophies (20). Myostatin blockade could increase the skeletal muscle mass, regardless of the type of muscu-

lar dystrophy. Antibody-mediated or myostatin propeptide-mediated myostatin blockade in *mdx* mice, a model for Duchenne type muscular dystrophy, ameliorates the pathophysiology and increases muscle strength (8, 9, 18) (Table 1). Crossing of myostatin knockout mice with *mdx* mice also attenuates severity of muscular dystrophy (21). The pathophysiologies of three models of limb-girdle muscular dystrophy, including δ-sarcoglycan-deficiency, caveolin-3 mutations and calpain-3-deficiency, are also ameliorated by myostatin blockade (10, 11, 22). However, myostatin elimination did not combat laminin-α2-deficiency in mice, but rather increased their postnatal mortality due to fat loss (12). Similarly, myostatin inhibition was not effective for prolonging the survival of LGMD2D model mice with mutations of α-sarcoglycan (11). However, since the expression by AAV-myostatin propeptide used in the study was extremely low, it is still possible that different mode of action, such as the use of neutralizing myostatin antibody could be beneficial for α-sarcoglycan deficiency (11).

Myostatin inhibition would increase the relative ratio of fast myofibers to slow myofibers. Exercise in myostatin-deficient cattle led to early exhaustion, which may have been caused by a decrease in the number of mitochondria (23). However, a decreased number of mitochondria associated with myostatin absence was specific for myostatin-knockout mice and not observed in myostatin-inhibitor-expressing transgenic mice (our unpublished observations). Thus, regulation of the number of mitochondria seems to depend on the way in which myostatin is inhibited. This observation suggests that myostatin inhibition by our follistatin-derived peptide would not decrease the number of mitochondria, although this aspect needs to be clarified in future studies.

Follistatin and FLRG are efficient myostatin blockers, and inhibit not only myostatin but also activins. We have developed a myostatin inhibitor derived from follistatin, designated FS I-I, that does not affect activin activity (17). FS I-I is capable of ameliorating the pathophysiology of *mdx* mice. It must be determined whether FS I-I affects other TGF-β-like ligands that regulate muscle fiber growth. Since transgenic expression of FS I-I is effective for treatment of *mdx* mice, FS I-I and related follistatin-derived myostatin inhibitors would join the list of potential therapeutic myostatin inhibitors.

Myostatin inhibitor peptides could be directly infused into muscular dystrophy patients. In addition, a delivery system using myogenic cells is also possible. Furthermore, myostatin inhibition could be combined with other therapeutic approaches. Myostatin inhibition is considered to be most effective when combined with gene correction or other ways of delivering dystrophin (24). In this sense, one advantage of myostatin inhibitor peptides is their application to combined therapy for muscular dystrophy. If cDNAs for myostatin inhibitor peptides can be expressed in myogenic stem cells, cell-mediated

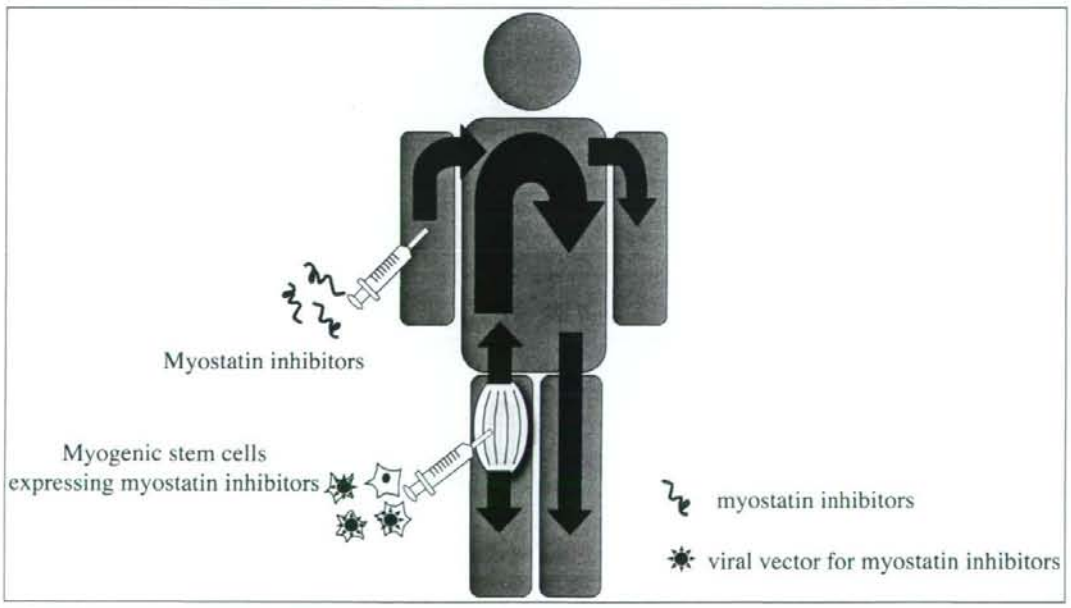

Figure 1. Potential delivery systems for myostatin inhibitors *in vivo*.

therapy with myostatin inhibition would become possible (Fig. 1). By using this method, defective genes such as dystrophin would be amended by myogenic stem cells. Alternatively, viral vectors containing myostatin inhibitor peptides could be combined with other possible therapies for muscular dystrophy, such as exon-skipping reagents or genes (24).

Studying the role of myostatin in tissues other than skeletal muscle is important to avoid the possible adverse effects of myostatin inhibition. In this respect, it is important to determine whether or not myostatin acts solely on skeletal muscles. Adipose tissues are affected by myostatin signaling. Reduction of adipose tissue mass is observed in myostatin-null mice. Whether myostatin directly acts on adipocytes or factors from hypertrophied skeletal muscle secrete factors affecting adipocyte remains to be determined.

Finally, ethical issues must be considered for use of myostatin inhibition. Athletes are already interested in myostatin for increasement of their muscle strength. There is a discussion that myostatin inhibition would be non-steroidal doping methods that are difficult to identify.

In summary, I have presented an outline of myostatin inhibition therapy for muscular dystrophy with emphasis on a myostatin inhibitor derived from follistatin. I hope that this novel therapeutic strategy will prove useful toward establishing realistic therapies for intractable diseases, such as muscular dystrophy.

Acknowledgements

This research was supported by grants from the Ministry of Health, Labour and Welfare.

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Acta Myologica • 2008; XXVII; p. 19-24

Caveolin-3 regulates myostatin signaling. Mini-review

Y. Ohsawa[1], T. Okada[1], A. Kuga[1], S. Hayashi[1], T. Murakami[1], K. Tsuchida[2], S. Noji[3], Y. Sunada[1]

[1] *Division of Neurology, Department of Internal Medicine, Kawasaki Medical School, 577 Matsushima, Kurashiki-City, Okayama 701-0192, Japan;* [2] *Division for Therapies against Intractable Diseases, Institute for Comprehensive Medical Science, Fujita Health University, 1-98 Dengakugakubo, Kutsukake-cho, Toyoake-City, Aichi 470-1192, Japan;* [3] *Department of Biological Science and Technology, Faculty of Engineering, The University of Tokushima, 2-1 Minami-Jyosanjima-cho, Tokushima-City, Tokushima 770-8506, Japan*

Caveolins, components of the uncoated invaginations of plasma membrane, regulate signal transduction and vesicular trafficking. Loss of caveolin-3, resulting from dominant negative mutations of caveolin-3 causes autosomal dominant limb-girdle muscular dystrophy (LGMD) 1C and autosomal dominant rippling muscle disease (AD-RMD). Myostatin, a member of the muscle-specific transforming growth factor (TGF)-β superfamily, negatively regulates skeletal muscle volume. Herein we review caveolin-3 suppressing of activation of type I myostatin receptor, thereby inhibiting subsequent intracellular signaling. In addition, a mouse model of LGMD1C has shown atrophic myopathy with enhanced myostatin signaling. Myostatin inhibition ameliorates muscular phenotype in the model mouse, accompanied by normalized myostatin signaling. Enhanced myostatin signaling by caveolin-3 mutation in human may contribute to the pathogenesis of LGMD1C. Therefore, myostatin inhibition therapy may be a promising treatment for patients with LGMD1C. More recent studies concerning regulation of TGF-β superfamily signaling by caveolins have provided new insights into the pathogenesis of several human diseases.

Key words: caveolin-3, limb-girdle muscular dystrophy 1C (LGMD1C), autosomal dominant rippling muscle disease (AD-RMD), myostatin, transforming growth factor-β (TGF-β)

Caveolins are primary components of caveolae

Caveolae, uncoated invaginations of the plasma membrane, are an abundant feature of many terminally differentiated cells, such as adipocytes, endothelial cells, and muscle cells. Caveolin family proteins, 21-24 kDa integral membrane proteins, are the principle components of caveolae, designated as caveolin-1, -2, and -3 (1, 2). Caveolin-1 and caveolin-2 are coexpressed and form heterooligomers in nonmuscle cells, whereas caveolin-3 is muscle specific and forms homooligomers in muscle cells (3, 4). De novo synthesized caveolins assemble to about 350 kDa oligomers in the endoplasmic reticulum, subsequently target to the plasma membrane via the trans-Golgi network, and play a crucial role in the formation of caveolae. These caveolin family proteins have been implicated in numerous cellular events including vesicular trafficking, lipid metabolism, and signal transduction (1-6). They directly bind to and regulate specific lipid and lipid-modified proteins including cholesterol, G-protein, G-protein coupled receptors, Src family kinase, Ha-Ras, and nitric oxide synthases (5-7). The interaction between caveolins and these molecules is mediated by a caveolin-binding motif on the target protein and a scaffolding domain in caveolin (7). The number of *in vitro* studies linking caveolins to signal transduction pathways has grown exponentially. To date, however, only a few studies have been concluded the exact roles of caveolins to signal transduction *in vivo* (3).

Dominant-negative mutations of caveolin-3 gene causes LGMD1C/AR-RMD

Many mutations in caveolin-3 gene have been detected in autosomal dominant limb-girdle muscular dystrophy (LGMD) 1C and autosomal dominant rippling muscle disease (AD-RMD) (8, 9). Mutations of the caveolin-3 gene cause a significant reduction in the cell surface level of caveolin-3 protein in a dominant-negative fashion and, to a lesser extent, mistargeting of the mutant caveolin-3 protein to the Golgi complex (8-10).

The loss of caveolin-3 by mutations of the caveolin-3 gene in LGMD1C/AD-RMD patients has resulted in subsequent abnormalities of caveolin-3-binding molecules. The

Address for correspondence: Yoshihide Sunada, MD, PhD, Division of Neurology, Department of Internal Medicine, Kawasaki Medical School, 577 Matsushima, Kurashiki-City, Okayama 701-0192, Japan. Fax +81 86 4641027. E-mail: ysunada@med.kawasaki-m.ac.jp

enzymatic activity of neuronal nitric oxide synthase, which is strongly suppressed by caveolin-3, increases in the skeletal muscles from a transgenic mouse model of LGMD1C and LGMD1C/AD-RMD patients (11, 12). Consistently, cytokine-induced NO production increases in C2C12 myoblast cells transfected with LGMD1C/AD-RMD-type mutant caveolin-3 compared to ones transfected with wild-type caveolin-3 (9). Src tyrosine kinase, a membrane tyrosine kinase whose activation regulates the balance between cell survival and cell death, is extremely activated and accumulates not in the plasma membrane but in the perinuclear region in cells transfected in LGMD1C/AD-RMD mutant caveolin-3 (13). Muscle-specific phosphofruktokinase, an enzyme of central importance in the regulation of glycolytic metabolism is also significantly reduced in cells transfected with LDMD1C/AD-RMD mutant caveolin-3 probably through ubiquitin-proteasomal degradation (14). Noteworthy also is the finding that dysferlin, a membrane-repair molecule deficient in LGMD2B/Miyoshi myopathy, mistargets to the cytoplasm from sarcolemma in skeletal muscle from LGMD1C/AD-RMD patients, probably due to the caveolin-3's delivery function to the correct targeting of plasma membrane (15-18).

Despite these findings, the underlying molecular mechanism leading to LGMD1C/AD-RMD in caveolin-3-deficient muscle remains to be elucidated.

Myostatin, a muscle-specific TGF-β superfamily member, is a therapeutic target of muscular dystrophy

Myostatin is a muscle-specific transforming growth factor (TGF)-β superfamily member and negatively regulates skeletal muscle growth and skeletal muscle volume (19). Overexpression of myostatin causes severe muscle atrophy, whereas targeted disruption of myostatin increases skeletal muscle mass in mice (19, 20). Like most members of the TGF-β superfamily, myostatin is synthesized as a precursor protein and undergoes proteolytic processing to generate an N-terminal prodomain and a biologically active, C-terminal disulfide-linked dimer (21). In the inactive state, the prodomain strongly inhibits the biological activity of the C-terminal dimer (22, 23), as do follistatin, and the follistatin-related gene (FLRG); which are collectively called natural inhibitors for myostatin (24). The circulating active form of myostatin directly binds to and phosphorylates the type II serine/threonine kinase receptor, namely activin receptor IIB (ActRIIB) (Fig. 1) (25). This, in turn, phosphorylates the type I serine/threonine kinase receptors, namely activin receptor-like kinase 4/5 (ALK4/5) at the plasma membrane (25-27). The activation of a heteromeric receptor complex consisting of phosphorylated type II and type I serine/threonine kinase receptors induces the phosphorylation of intracellular effectors, receptor-regulated Smads (R-Smads), namely Smad2/3 (26, 27). Phosphorylated R-Smads translocate to the nucleus from the cytoplasm, where it regulate the transcription of specific target genes inducing skeletal muscle atrophy (26-28).

Notably, administration of a blocking antibody against myostatin, myostatin vaccine, and myostatin prodomain, or genetic introduction of a follistatin-derivative ameliorates the pathophysiology of dystrophin-deficient *mdx* mice (29-32). In addition, a blocking antibody against myostatin improves the condition of young model mice with δ-sarcoglycan-deficient LGMD2F (33). An adeno-associated virus (AAV)-mediated myostatin prodomain has ameliorates the pathology of calpain-3-deficient LGMD2A model mice (34). Therefore, myostatin inhibition through different strategies has recently come to be considered for a therapeutic option for muscular dystrophies. However, the precise molecular mechanism by which myostatin inhibition improves the above dystrophic skeletal muscle is not fully understood; i.e. the molecular interaction of myostatin and the dystrophin-glycoprotein complex is unknown.

Caveolin-1 regulates TGF-β superfamily signaling *in vitro*

Recently, caveolin-1 has drawn attention as a regulator of TGF-β superfamily signaling. Caveolin-1 binds to and suppresses activation of the type I receptor of TGF-β1, which induces growth arrest in nonmuscle cells (35). Consistently, the binding affinity of caveolin-1 with type I TGF-β1 receptor decreases after stimulation with TGF-β1. In addition, caveolin-1 associates with the type II receptor of TGF-β1 (36-38). Caveolin-1 also facilitates ligand–bound TGF-β1 receptors internalization and degradation via the formation of endocytic vesicles with ubiquitin-ligase (39, 40). In addition, caveolin-1 interacts with type II and type I receptors of bone morphogenic proteins (BMPs) *in vivo* (41). These findings indicate that caveolin-1 regulates TGF-β superfamily signaling, including TGF-β1 and BMPs, at its receptor level.

Caveolin-3 suppresses myostatin signaling through its type I receptor *in vitro*

Upon consideration of molecular analogy and tissue distribution, we hypothesized that caveolin-3 inhibits myostatin signaling in a similar manner to that of inhibition

of caveolin-1 to multiple TGF-β superfamily signaling in nonmuscle cells. We found several caveolin-3 binding motifs (7); ϕXϕXXXXϕXXϕX, where ϕ indicates aromatic or aromatic-like amino acids in the cytoplasmic kinase domain of type I serine/threonine myostatin receptors, ALK4/5 (42). Therefore, we cotransfected caveolin-3 and these type I myostatin receptors in COS-7 monkey kidney cells and found that caveolin-3 colocalized with type I myostatin receptor. Immunoprecipitation and subsequent immunoblot analysis revealed that caveolin-3 associates with the type I myostatin receptor. In addition, phosphorylation level of the type I myostatin receptor decreased with the addition of caveolin-3 in cells cotransfected with constitutively active type I receptor and caveolin-3. Moreover, caveolin-3 eventually suppressed subsequent intracellular myostatin signaling; the phosphorylation level of an R-Smad of myostatin, Smad2 as well as the transcription level of the Smad-sensitive $(CAGA)_{12}$-reporter gene. Therefore, caveolin-3 suppresses the myostatin signal at its type I receptor level, in a similar manner to caveolin-1 for TGF-β1 signaling *in vitro*.

Caveolin-3 deficient muscles exhibit enhanced intracellular myostatin signaling

We previously generated transgenic (Tg) mice overexpressing mutant caveolin-3 (CAV-3^{P104L}) to develop a mouse model of LGMD1C/AD-RMD (11). The skeletal muscle phenotype of the transgenic mice showed severe myopathy with loss of caveolin-3. To determine whether caveolin-3 regulates myostatin signaling *in vivo*, we generated and characterized the double-transgenic mice showing myostatin deficiency and myostatin inhibition. Heterozygous mating of mutant caveolin-3 Tg mice with other Tg mice overexpressing myostatin prodomain ($MSTN^{Pro}$) (43), a potent inhibitor of myostatin signaling, gave rise to mice with four distinct phenotypes: wild-type, mutant caveolin-3 Tg, mutant MSTN Tg, and double-mutant Tg (CAV-3^{P104L}/$MSTN^{Pro}$). Growth curves revealed that the double-mutant Tg mice were significantly larger than the mutant caveolin-3 Tg mice and similar in size to the wild-type mice beginning at 6 weeks until 16 weeks of age (42). The muscle atrophy seen in the mutant caveolin-3 Tg was reversed in the double-mutant Tg with increased myofiber size and myofiber number. Thus, myostatin inhibition reverses caveolin-3-deficient muscular atrophy *in vivo*.

Caveolin-3-deficient muscle from mutant caveolin-3 Tg mice showed hyperphosphorylation of an R-Smad of myostatin, Smad2 and significant upregulation of a myostatin target gene, p21. These *in vivo* findings were consistent with our *in vitro* study in which caveolin-3 suppresses myostatin signaling. In the double-mutant Tg mouse, the levels of phospho-Smad2 and p21 gene expression were significantly reduced compared to those in the mutant caveolin-3 Tg mice and were similar to those in the wild-type mice. Thus, myostatin inhibition by genetic introduction of myostatin inhibitor normalized enhanced myostatin signaling and also reversed muscular phenotype in the caveolin-3 deficient mouse.

Myostatin inhibition therapy reversed muscular atrophy in caveolin-3 deficiency

We injected a soluble form of the extracellular domain of type II myostatin receptor, ActRIIB, which can inhibit myostatin-its type II receptor binding (25, 44), into the mutant caveolin-3 Tg mice to develop myostatin inhibition through its type II receptor as a therapeutic strategy for patients with LGMD1C. Intraperitoneal injection of soluble ActRIIB four times significantly increased skeletal muscle mass and reversed myofiber hypotrophy accompanied with suppression of Smad2 phosphorylation and downregulation of p21. This finding, therefore, suggests that myostatin inhibition therapy may be a reasonable and promising therapy for caveolin-3-deficient muscular dystrophy associated with enhanced myostatin signaling.

Conclusions and prospective for future research

Caveolin-3 has been considered to regulate numerous signal pathways for maintaining the normal integrity of skeletal muscles, but the *in vivo* significance of signal alterations by loss of caveolin-3 in the pathogenesis of LGMD1C/AD-RMD has not been well delineated. As reviewed herein, caveolin-3 regulates myostatin signaling *in vitro*, and thus disrupted interaction between caveolin-3 and myostatin could contribute to the pathogenesis of caveolin-3-deficient muscular dystrophy (Fig. 1).

We could not conclude that activated intracellular signaling molecules, hyperphosphorylation of an R-Smad, Smad2, and upregulation of p21 in the caeveolin-3 deficient skeletal muscle result simply from enhanced myostatin signaling by loss of caveolin-3, because the myostatin prodomain or the soluble myostatin receptor suppresses not only myostatin, but also other TGF-β ligands including growth and differentiation factor 11 (GDF11) (22, 25, 44, 45). In fact, evidence of an unknown TGF-β ligand exists in the form of a similar negative regulator of muscle mass like myostatin (45, 46). Thus TGF-β ligands other than myostatin also could be

involved in the pathogenesis of caveolin-3 deficieny via the Smad2-p21-mediated pathway. Crossing of mutant caveolin-3 mice with myostatin-null mice is a prospective project for obtaining straightforward evidence that hyperphosphorylation of Smad2 and upregulation of p21 in caveolin-3-deficient muscles is the simple result of enhanced myostatin signaling.

More recent studies have shown to be caveolins as an exact negative regulator of TGF-β superfamily signaling because the loss of caveolins has play important roles in the pathogenesis of human disorders. Mutations of the caveolin-1 gene or downregulation of caveolin-1 protein have been detected in some sporadic breast cancers (47) and epithelial cells derived from caveolin-1 null mice have shown hyperphosphorylation of Smad2 and epithelial mesenchymal transition, corresponding to premalignant status (48). In addition, loss of caveolin-1 has been strongly associates with idiopathic pulmonary fibrosis (49, 50). Caveolin-1 protein has been found to be reduced in the lung tissue from patients with idiopathic pulmonary fibrosis. TGF-β1-induced extarcellular matrix production, which is indicative of fibrosis, significantly increases in

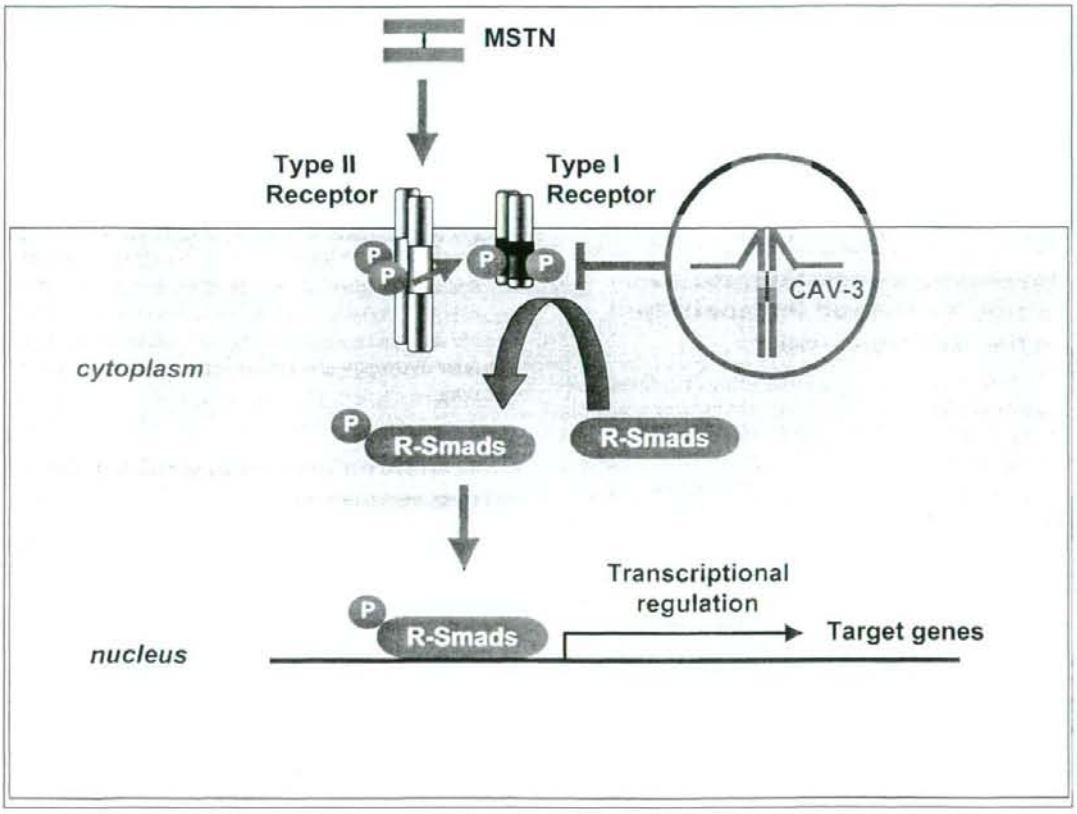

Figure 1. Putative scheme of the regulation of myostatin signaling by caveolin-3. Myostatin (MSTN) signaling is propagated through the myostatin receptor, a heteromeric complex consisting with transmembrane receptor serine/threonine kinases. Myostatin binds to and phosphorylates its type II serine/threonine kinase receptor (Type II Receptor). Subsequently, its type I serine/threonine kinase receptor (Type I Receptor) is phosphorylated by Type II Receptor and is recruited into the heteromeric complex, which in turn phosphorylates receptor-regulated Smads (R-Smads), a family of transcription factor controlling the expression of specific target genes. Caveolin-3 (CAV-3) binds to and suppresses activation of the Type I Receptor of MSTN at the plasma membrane and suppresses intracellular myostatin signaling, including phosphorylation of R-Smads and transcription of specific target genes. Loss of caveolin-3 resulting from dominant negative mutations of the caveolin-3 genes in patients with LGMD1C could enhance intracellular myostatin signaling, and thereby result in muscle mass reduction. Type II Receptor, ActRIIB; Type I Receptor, ALK4/5; R-Smads, Smad2/3. P indicates phosphorylation.

primary fibroblasts isolated from patients with idiopathic pulmonary fibrosis. Moreover, retroviral introduction of caveolin-1 ameliorates bleomycin-induced lung fibrosis in mice. Together with this review, it may be concluded that aberrant TGF-β superfamily signaling by loss of caveolins participate in the pathogenesis of some human diseases, including LGMD1C/AD-RMD, breast cancer, and idiopathic pulmonary fibrosis.

Myostatin inhibition therapy is effective, to some extent, with mouse models of several types of muscular dystrophies (29-34). Further investigation is needed to determine which types of myostatin inhibition therapy could be applied and to clarify the molecular mechanism by which myostatin-inhibition improves muscular dystrophy for prospective treatment of patients with muscular dystrophy. As reviewed herein, myostatin inhibition may be a potent therapy for caveolin-3-deficient muscular dystrophy with enhanced myostatin signaling.

Acknowledgments

We are grateful to Ms. N. Akazawa for critical reading of the manuscript. This work was supported by a Research Grant (14B-4) for Nervous and Mental Disorders from the Ministry of Health, Labor and Welfare; Grants (15131301 and 17231401) for Research on Psychiatric and Neurological Diseases and Mental Health from the Ministry of Health, Labor and Welfare of Japan and from the Japan Society for the Promotion of Science (JSPS) KAKENHI (14370212).

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