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LDB3 splicing abnormalities are specific to skeletal muscles of patients with myotonic dystrophy type 1 and alter its PKC binding affinity

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ABSTRACT

Myotonic dystrophy type 1 (DM1) is caused by transcription of CUG repeat RNA, which causes sequestration of muscleblind-like 1 (MBNL1) and upregulation of CUG triplet repeat RNA-binding protein (CUG-BP1). In DM1, dysregulation of these proteins contributes to many aberrant splicing events, causing various symptoms of the disorder. Here, we demonstrate the occurrence of aberrant splicing of LIM domain binding 3 (*LDB3*) exon 11 in DM1 skeletal muscle. Exon array surveys, RT-PCR, and western blotting studies demonstrated that exon 11 inclusion was DM1 specific and could be reproduced by transfection of a minigene containing the CTG repeat expansion. Moreover, we found that the *LDB3* exon 11-positive isoform had reduced affinity for PKC compared to the exon 11-negative isoform. Since PKC exhibits hyperactivation in DM1 and stabilizes CUG-BP1 by phosphorylation, aberrant splicing of *LDB3* may contribute to CUG-BP1 upregulation through changes in its affinity for PKC.

Introduction

Myotonic dystrophy (DM) is the most common cause of adult-onset muscular dystrophy. The symptoms of DM are systemic, affecting multiple organs, such as the skeletal muscle (myotonia, muscle wasting), heart (arrythmia, conduction defects, dilated cardiomyopathy), brain (dementia, sleeplessness), and endocrine system (insulin resistance) (Harper, 2001). The most common form of the disease is DM type 1 (DM1), an autosomal dominant inherited disorder caused by a CTG repeat expansion in the 3′-untransrated region (UTR) of the *DMPK* gene. The etiology of DM1 is mainly the gain of function achieved by a toxic mRNA (Osborne and Thornton, 2006; Ranum and Cooper, 2006). RNA containing expanded CUG repeats sequesters an important splicing factor, muscleblind-like 1 (MBNL1). On the other hand, another splicing factor, CUG triplet repeat RNA-binding protein (CUG-BP1), is overexpressed in conjunction with expression of the toxic repeat-containing RNA. Dysregulation of these 2 splicing factors is thought to cause aberrant splicing events in various genes and results in systemic symptoms observed in patients with DM1 (Osborne and Thornton, 2006; Ranum and Cooper, 2006).

To date, 28 aberrantly spliced exons have been identified in striated muscle. Additionally, exon array analysis of aberrant splicing events identified 2 previously described DM1-specific events (Yamashita et al., 2012). Among these events, aberrantly spliced LIM domain binding 3 (*LDB3*) is known to play an integral role in the interaction between the muscular Z-line and α-actinin-2 (Faulkner et al., 1999; Zhou et al., 1999). Moreover, mutations or depletions in *LDB3* are associated with dilated cardiomyopathy and myofibrillar myopathy (Arimura et al., 2004, 2009; Cheng et al., 2011; Griggs et al., 2007; Vatta et al., 2003; Xing et al., 2006; Zheng et al., 2009; Zhou et al., 2001), which are closely related to the cardiac symptoms of DM.

LDB3 interacts with protein kinase C (PKC) through its C-terminal LIM domains (Zhou et al., 1999). In DM1, PKC is inappropriately activated and phosphorylates CUG-BP1, leading to its overexpression (Kuyumcu-Martinez et al., 2007). Therefore, we speculated that aberrant splicing of *LDB3* may change the affinity of the LDB3 protein for PKC and contribute to variations in PKC activation. In the current study, we investigated the specificity of aberrant splicing of *LDB3* exon 11 to DM1 skeletal muscle and the effects of inclusion of exon 11 on the affinity of the LDB3 long isoform for PKC.

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Materials and methods

Patient samples

DM1 skeletal muscles were previously biopsied for diagnostic purposes ($n = 8$, at Aichi Medical University, details given in Table 1). All the samples were diagnosed with Southern blot analysis to confirm the approximate repeat numbers. Three normal control muscle specimens showing no pathological abnormalities were used. Disease controls were also previously biopsied for diagnostic purposes, including samples from patients with amyotrophic lateral sclerosis (ALS), spinal and bulbar muscular atrophy (SBMA), Becker type muscular dystrophy (BMD), Duchenne type muscular dystrophy (DMD), and polymyositis/dermatomyositis (PM/DM). Detailed information about the biopsy samples is given in Table 1. High-molecular-weight DNA was extracted by the conventional proteinase K and phenol chloroform method. We determined the CTG repeat numbers at the 3′ UTR of the *DMPK* gene by Southern blotting (Table 1). Statistical analysis of differences between the samples was performed by Student's *t*-test.

All experiments were approved by the Institutional Review Board of Nagoya University Graduate School of Medicine and Aichi Medical University. The samples were used for the current studies after appropriate informed consent was obtained from all patients.

Skeletal muscle RT-PCR

We searched for aberrant splicing in DM1 skeletal muscle using a GeneChip Human *Exon* 1.0 ST Array according to our previously described methods (Yamashita et al., 2012). Total RNA was extracted with the RNeasy Mini Kit (Qiagen, Valencia, CA, USA), according to the manufacturer's protocol. We reverse transcribed 100 ng of total RNA using SuperScript II (Invitrogen, Carlsbad, CA, USA) with oligo $(dT)_{15}$ primers. Then, we performed PCR using the cDNA as a template, 2X Master Mix (Qiagen), and 0.2 μM flanking primers on exon 8 and exon 12 (FWD: 5′-GGACCTTGCCGTAGACA, REV: GTAGACAGAAGGCCGGAT). The Multiplex RT-PCR was performed using Multiplex PCR Kit (Qiagen). Primers were as follows. For long isoform (FWD on exon 16: 5′-GTCCCA TTCCCATCTCCAC, REV on exon 17: GCCCTCACTGTAGCTGGTGT); short isoform (FWD on exon 9: 5′-CGAAGGTCAAGGGAAAGGTT, REV on exon 10: TTAACATTAAGGATTTGGGCTGA). We used 3 primers as follows for separating each LDB3 isoform: exon 3 (FWD: 5′-GTCCCATTCCCATCTC CAC), exon 10 (REV: AAACGTGGGCTGTTACGTTC), exon 12 (REV: GGTA GACAGAAGGCCGGATG). Electrophoresis was performed using ethidium bromide-stained 2.5% agarose gels.

LDB3 exon 11 minigene assay

First, we constructed the *LDB3* exon 11 minigene, which consisted of fragments containing an end region of exon 9 (34 bp, full length 37 bp) and downstream intron of exon 9 (1552 bp), an upstream intron of exon 11 (1357 bp) and exon 11 (189 bp), downstream intron of exon 11 (3185 bp, full length), and a part of exon 12 (73 bp, full length 146 bp; Fig. 1A). Each fragment was PCR-amplified using human genomic DNA as a template with KOD -plus- (Toyobo, Tokyo, Japan). Then, each fragment was ligated into pcDNA3.1 (Invitrogen) using the *Nhe*I/*Not*I/*Xho*I site. Constructing a minigene containing the full length intron around exon 11 was difficult since the length of the upstream intron was too long (13,959 bp). One microgram of the minigene was cotransfected into C2C12 cells (60% confluent) in 6-well plates with DMPK, DT960, GFP-CUGBP1, or GFP-MBNL1 40 kDa using Lipofectamine 2000 (Invitrogen). Cells were harvested, and total RNA was extracted with the RNeasy Mini Kit (Qiagen). RT-PCR was performed using a forward primer targeting the pcDNA3.1 site (FWD: AGAAGCTCTGCGAAGGTC AA) and a reverse primer targeting *LDB3* exon 12 (REV: CAACAGATGG CTGGCAACTA).

Table 1
Skeletal muscle samples used for RT-PCR and western blotting.

Samples	Age/sex	Biopsy site	# of DM1 expanded repeat
DM1 1[a]	58/F	lBi	1450 (WBC[b])
DM1 2[a]	40/M	lBi	4500 (muscle)
DM1 3[a]	38/F	lBi	4230 (muscle)
DM1 4	30/F	lBi	4000 (muscle)
DM1 5	52/F	lBi	3430 (muscle)
DM1 6	44/M	lB	4300 (muscle)
DM1 7	22/F	rVL	3830 (muscle)
DM1 8	47/M	lBi	3770 (muscle)
ALS 1	58/M	rVL	
ALS 2	45/F	lVL	
SBMA 1	55/M	lBi	
BMD 1	27/M	lB	
BMD 2	35/M	lBi	
DMD 1	1/M	lVL	
DMD 2	7/M	lBi	
PM/DM 1	51/F	rVL	
PM/DM 2	52/F	rVL	
FSH 1	19/M	lBi	
FSH 2	41/M	lBi	
IBM 1	75/M	lQ	
IBM 2	68/F	rTA	
NC 1	51/F	lVL	
NC 2[a]	77/M	unknown	
NC 3[a]	71/F	unknown	

DM1: myotonic dystrophy type 1; ALS: amyotrophic lateral sclerosis; SBMA: spinal bulbar muscular atrophy; BMD: Becker type muscular dystrophy; DMD: Duchenne type muscular dystrophy; PM/DM: polymyositis/dermatomyositis; FSH: facioscapulohumeral muscular dystrophy; IBM: inclusion body myositis; NC: normal control.
lBi: left biceps; rVL, lVL: right or left vastus lateralis; lQ: left quadriceps; rTA: right tibias anterior.
[a] Only RNA but not protein samples were available.
[b] White blood cells.

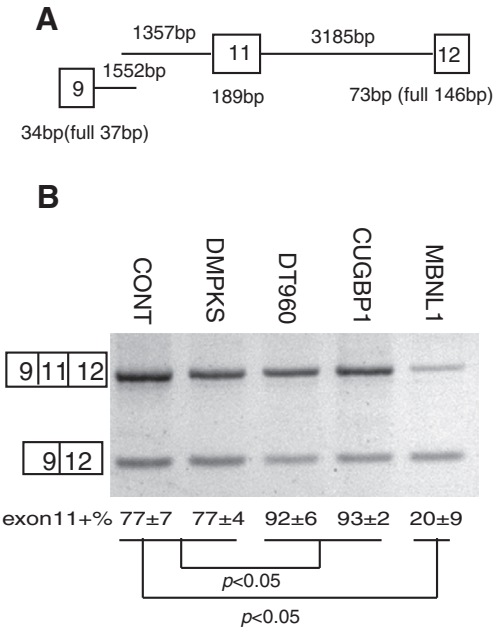

Fig. 1. CTG repeat expansion caused exon 11 inclusion. A. Schematic representation of the LDB3 exon11 minigene containing 34 bp of exon 9, 1552 bp downstream of exon 9, 1357 bp upstream of exon 11, exon 11 (189 bp), 3185 bp downstream of exon 11, and 73 bp of exon 12. B. RT-PCR of the LDB3 exon 11 minigene transfection assay. Average rates of exon 11-containing PCR products are shown at the bottom of the figure, and ± indicates the SE (standard error, $n = 3$). Lanes represent minigenes transfected with minigene only, DMPKS (the DMPK construct with no repeat expansion), DT960 (the DMPK construct with the 960 CTG repeat expansion), CUGBP1, and MBNL1.

Western blotting of skeletal muscle protein

Proteins were extracted from human skeletal muscle biopsies. Tissues were homogenized with a polytron in lysis buffer (50 mM Tris–HCl, pH 7.5, 0.15 M NaCl, 1 mM EDTA, 1% NP-40, and 1 protease inhibitor cocktail tablet). Homogenates were centrifuged (20 min, 12,000 rpm), and the pellet was discarded. Protein concentrations were determined by the Bradford assay (Bio-Rad, Hercules, CA, USA). Additional samples were added to confirm splice isoform specificity to DM1. Total proteins extracts (50 μg) were separated on 12% SDS-PAGE gels and transferred onto nitrocellulose membranes. The membranes were blocked with 5% nonfat dry milk in TBS-T buffer (Tris buffer saline plus 0.1% Tween-20). Incubation with primary antibodies (anti-LDB3 antibodies, ab40840, Abcam) was performed overnight at 4 °C at a concentration 3:50,000 in TBS-T buffer, followed by incubation for 1 h with secondary antibodies (1:5000, ExactaCruz, Santa Cruz Biotechnology, Santa Cruz, CA, USA) in 1% skim milk.

LDB3 and PKC isoform construction

The LDB3 long isoform (exon 11-negative or exon 11-positive) and short isoform were LA-PCR amplified from human cDNA and inserted into the *p3xFLAG*-CMV-7.1 vector (Sigma Aldrich, St. Louis, MO, USA) at the *Bgl*II-*Kpn*I restriction site. PKCα, PKCβ, and PKCε were also LA-PCR amplified from commercial templates and inserted into myc-tagged vectors (gift from Kozo Kaibuchi, Nagoya University Graduate School of Medicine).

Immunoprecipitation assay

Two micrograms of FLAG-tagged LDB3 short isoform, long exon 11-negative isoform, or long exon 11-positive isoform was cotransfected with 2 μg of myc-tagged PKCα, PKCβ, or PKCε into COS-7 cells. Twenty-four hours later, proteins were extracted with lysis buffer (as above) and centrifuged (20 min, 12,000 rpm). Immunoprecipitation was performed using anti-myc-Tag agarose (mouse IgG1, MBL), according to the manufacturer's protocol. Cell lysates and immunoprecipitated products were separated on 10% SDS-PAGE gels, transferred to membranes, and incubated with primary antibodies (anti-myc 9E10, 1:2000, Abcam; anti-FLAG M2, 1:5000, Sigma). ECL-anti-mouse IgG (1:5000, GE Healthcare) was used as the secondary antibody. The binding affinity of FLAG-tagged LDB3 isoforms for myc-tagged PKCs was calculated by dividing the intensity of anti-FLAG with the intensity of anti-myc in western blotting of immunoprecipitated products.

Results

Aberrant splicing of exon 11 was specific to DM1

LDB3 was identified as having an aberrantly spliced exon by our previous exon array studies (Yamashita et al., 2012). Exon 4 and exon 11 (previously termed as exon 7 by us) were both included specifically in DM1. In this paper, exon 11 is identical to exon 7 in our previous report, in which the exon number was based on NM_007078.2 in the NCBI RefSeq database. Exon 11 is the 11th exon when all exons in all the transcripts annotated by ENSEMBL release 68 are taken into account. The LDB3 isoform containing exon 11 was observed in more than 50% of mRNA in DM1 samples, but less than 20% in control samples and samples from other diseases. Differences between the DM1 and normal control groups and between the DM1 and disease control groups were statistically significant ($p < 0.05$).

Aberrant splicing was caused by expansion of CTG repeats

To assess which factors contributed to abnormal splicing of *LDB3* exon 11, we performed overexpression assays by cotransfecting C2C12 cells with an *LDB3* exon 11 minigene and other constructs. DT960 (containing 960 CTG repeats and expressing 960 CUG without protein expression) promoted exon 11 inclusion compared to DMPK (including no repeat, Fig. 1). Therefore, we assumed that CTG repeat expansion itself was a contributing factor for exon 11 inclusion. In addition, CUG-BP1 overexpression promoted exon 11 inclusion, while MBNL1 overexpression decreased exon 11 inclusion.

An isoform shift was also observed at the protein level

Although aberrant splicing of *LDB3* exon 11 was confirmed at the mRNA level, its effect on protein expression was not clear. Alternative splicing of exons 5, 6, and 11 as well as alternative transcription termination predicts six LDB3 isoforms (Fig. 2A). All isoforms are either registered in the RefSeq database or in a previous report (Faulkner et al., 1999). Western blot analyses using skeletal muscle biopsy samples and antibodies targeting LDB3 revealed that the exon 11-positive isoform was expressed at significantly higher levels in DM1 muscles than in control muscles and muscle samples from patients with other neuromuscular disorders (Fig. 2B). These results demonstrated that DM1 samples clearly contained more of the exon 11-positive LDB3 isoform than samples from other disorders. Western blot analysis also revealed that more long isoform (78 kDa and 84 kDa) was expressed than short isoform (32 kDa) in DM1 (Fig. 2B). The difference was statistically significant ($p < 0.05$). We then analyzed the expression of the short and long isoforms with multiplex RT-PCR (Fig. 2C). The long isoform was also predominant in DM1 with statistical significance ($p < 0.05$).

To discriminate cardiac and skeletal isoforms, we performed multiplex RT-PCR with 3 primers with the forward primer on exon 3, the reverse primer on exon 10 (the final exon of short isoform), and on exon 12 (Fig. 2D). The exon 5-6-8-9-11-positive product was predominant in DM1. Importantly, the exon 4 positive isoform seemed to be not mainly expressed in DM1. We generated *LDB3* clones using DM1 cDNA as a template. Among 10 long isoform clones, no exon 4-positive ones were found (data not shown).

We found the exon 5-6-negative-7 (6a)-positive isoform (bottom band) only in DM1 samples as previously reported (Fig. 2D).

Aberrant splicing changed PKC-binding affinity

Because LDB3 has been shown to bind to PKC, we next investigated differences in the binding affinities of various LDB3 isoforms for PKC (Figs. 2A and 3). Importantly, the amounts of FLAG-tagged LDB3 isoforms and myc-tagged PKC isoforms expressed following transfection were similar, and myc-tagged PKC isoforms were immunoprecipitated with similar efficiencies. We observed that exon 11-positive LDB3 exhibited about 50% reduced binding affinity for PKCα and PKCβ compared to exon 11-negative LDB3. No significant difference was observed in terms of PKCε binding affinities between exon 11-negative and -positive LDB3 (Fig. 3, bottom panels).

Discussion

The importance of LDB3 and the specificity of aberrant exon 11 splicing to DM1

In DM1, many structural proteins in muscle fibers are aberrantly spliced, including α-dystrobrevin (Nakamori et al., 2008), BIN1 (Fugier et al., 2011), CAPN3 (Lin et al, 2006), DMD (Nakamori et al., 2007), DTNA, LDB3 (Lin et al., 2006; Yamashita et al., 2012), MYOM1 (Koebis et al., 2011), MYH14 (Rinaldi et al., 2012), NRAP (Lin et al., 2006), PDLIM3 (ALP) (Ohsawa et al., 2011), TNNT2, TNNT3 (Philips et al., 1998), and TTN (Lin et al., 2006; Yamashita et al., 2012). Among these, aberrant splicing of *BIN1* exon 11 has been shown to be associated with T tubule alterations and muscle weakness *in vivo* and *in vitro*.

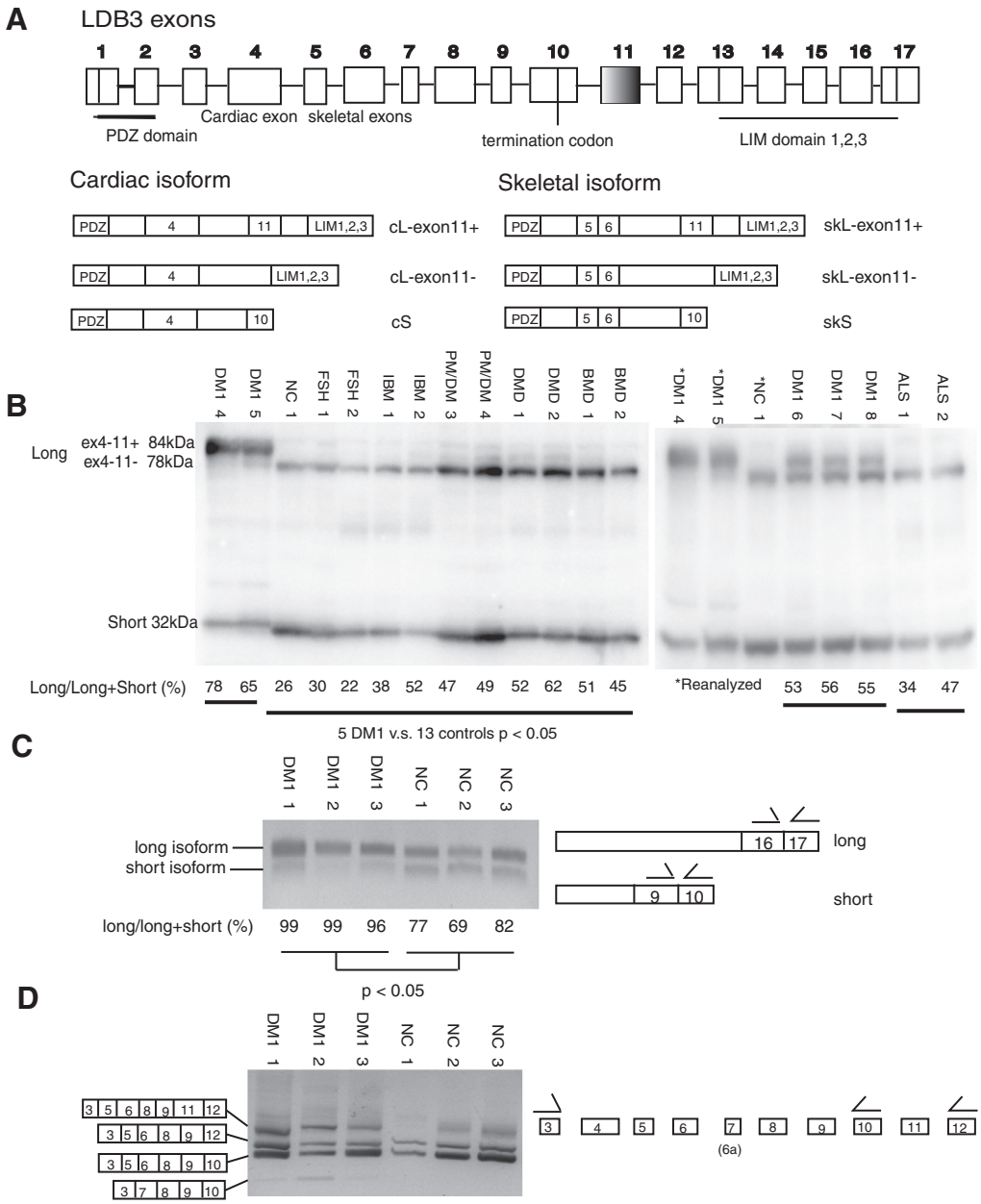

Fig. 2. Exon 11 splicing abnormalities were present at the protein level and specific to DM1. *LDB3* long isoforms were predominantly expressed in DM1, and exon 4 was rarely expressed. A. Schematic diagram of LDB3 isoforms. Cardiac isofoms included exon 4, whereas skeletal isoforms included exons 5 and 6. cL-exon11+: cardiac long isoform with exon 11, cL-exon11-: cardiac long isoform without exon11, cS: cardiac short isoform, skL-exon11+: skeletal muscle long isoform with exon 11, skL-exon11-: skeletal muscle long isoform without exon 11, skS: skeletal muscle short isoform. B. Western blotting of skeletal muscles using anti-LDB3 antibodies. The upper band for DM1 was 84 kDa containing exon 11 and the lower band was around 78 kDa, without exon 11. The short isoform was 35 kDa ($n = 5$ DM1 samples). The difference of long/(long + short) ratio was statistically significant between DM1 and other samples ($p < 0.05$). C. Multiplex RT-PCR to detect long and short isoform. The difference between DM1 and normal control was statistically significant ($p < 0.05$). D. Multiplex RT-PCR to detect exon expression from exon 3 to exon 12. Exon 5 and 6-positive isoforms were predominantly expressed in DM1.

However, the roles of aberrant splicing of other muscular structural proteins in the pathogenesis of DM1 remain to be elucidated.

In this study, we focused on the aberrant splicing of LDB3 because 1) LDB3 exists in Z-lines, key sections of muscle fibers required for contraction (Faulkner et al., 1999; Zhou et al., 1999); 2) LDB3 interacts with PKC (Zhou et al., 1999), which is upregulated in DM1 and stabilizes CUG-BP1 (Kuyumcu-Martinez et al., 2007); and 3) mutations or depletion of LDB3 result in cardiac symptoms similar to those observed in patients with DM1 (Arimura et al., 2004, 2009; Bhakta et al., 2004; Cheng et al., 2011; Griggs et al., 2007; Groh et al., 2008; Vatta et al., 2003; Xing et al., 2006; Zheng et al., 2009; Zhou et al., 2001). Our study demonstrated that abnormal inclusion of *LDB3* exon 11 was specific to DM1. Indeed, although the inclusion of *LDB3* exon 11 in DM1 has been reported previously (Lin et al., 2006; Vihola et al., 2010), the specificity of this missplicing event to DM1 had not been fully assessed.

Inclusion of LDB3 exon 4 in DM1 skeletal muscles

LDB3 is known to have long (over 70 kDa) and short (about 32 kDa) isoforms (Huang et al., 2003). Although the long isoform observed in DM1 was reported to contain not only exon 11 but also exon 4, which is specific to cardiac tissue (Lin et al., 2006; Vihola et al., 2010), our efforts to sequence 10 clones following the amplification of *LDB3* cDNA from DM1 skeletal muscles demonstrated that all clones including exon 11 did not contain exon 4, but did contain exons 5 and 6, which are specific to skeletal muscle. RT-PCR also revealed that exon 4

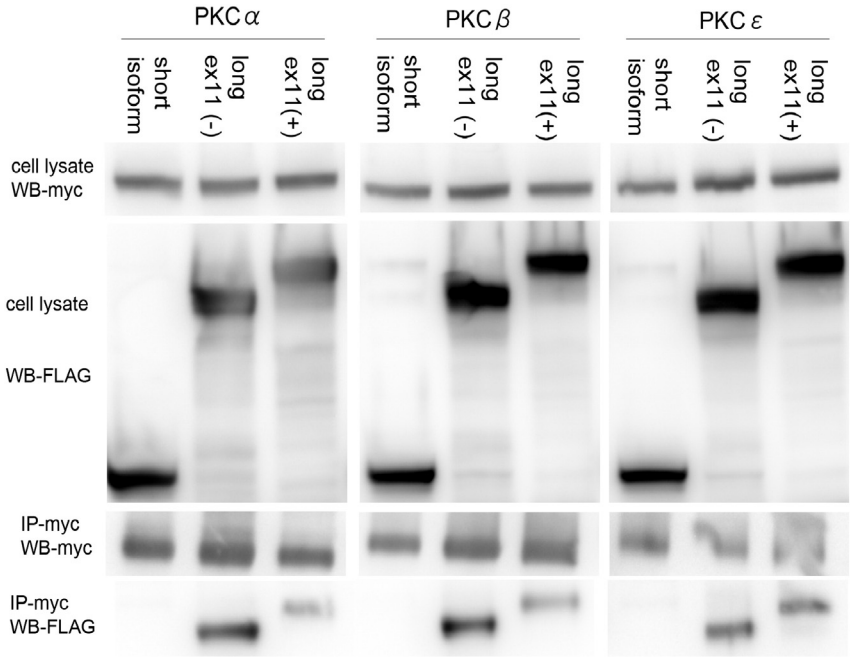

Fig. 3. The LDB3 exon 11 + isoform exhibited reduced binding affinity to PKCα and PKCβ. Cotransfections with LDB3 isoforms and PKC isoforms were performed, and whole cell lysates were harvested. Immunoprecipitation was then performed using these whole cell lysates. The ratio of FLAG/myc was calculated in order to quantify the binding affinity of FLAG-tagged LDB3 isoforms with myc-tagged PKCs.

expression was not predominant in DM1 (Fig. 2D). This suggested that the uppermost band (84 kDa) observed in western blotting represented the exon 11-positive long isoform, while the 78-kDa band represented the exon 11-negative long isoform, and the 32-kDa band represented the exon 11-negative short isoform, consistent with data presented in previous literature (Vihola et al., 2010). In any case, our data clarified that the abnormal exon 11-positive long isoform was overexpressed in DM1 skeletal muscles.

In a previous study, we reported that exon 4 inclusion was specific to DM1 muscles (Yamashita et al., 2012). Although this is somewhat contradictory to the current study, it is possible that the exon 4-positive long isoform (i.e., the cardiac exon) may be very rare compared to the isoform containing exons 5 and 6 (skeletal exons). On the other hand, inclusion of *LDB3* exon 4 may also contribute to the pathogenesis of DM1. Interestingly, mutations in *LDB3* exon 4 have been shown to reduce the affinity of LDB3 for PGM1 and are associated with dilated cardiomyopathy (Arimura et al., 2009). Thus, inclusion of exon 4 in LDB3 from DM1 skeletal muscles may change the affinity of LDB3 for PGM1 and lead to muscular structural changes and weakness.

Exon 7 inclusion and skipping of exons 5 and 6

We also confirmed exon 7(6a) inclusion and skipping of exon 5 and 6 in DM1 (Fig. 2D) as previously described (Machuca-Tzili et al., 2006). The pathogenic role of this mis-splicing also remains to be elucidated.

Factors influencing the regulation of exon 11 splicing

In this study, we confirmed that CTG repeats caused *LDB3* exon 11 inclusion. Moreover, overexpression of MBNL1 led to exclusion of exon 11, while overexpression of CUG-BP1 led to inclusion of exon 11. Thus, although another report suggested that MBNL1 is the only factor regulating the splicing of *LDB3* exon 11 (Kalsotra et al., 2008), CUG-BP1 may also be involved in the regulation of exon 11 splicing (Fig. 1). The antagonistic effect of MBNL1 overexpression was more prominent than that of CTG repeat or CUG-BP1 overexpression. This may be due to the basal high inclusion (about 70%) of exon 11 minigene, and the existence of 11 candidate MBNL1 YGCY binding sites (Goers et al., 2010) (7 and 4 in the bilateral flanking 500 bp upstream and downstream introns, respectively, from SpliceAid: http://www.introni.it/splicing.html).

LDB3 isoforms and PKC binding affinity

We next considered the functional significance of *LDB3* exon 11 inclusion in the pathogenesis of DM1. The long isoform of *LDB3* interacts with PKC through its LIM domain, while the short isoform does not. Although exon 11 is not a part of LIM domain, its existence may alter the binding affinity of the LDB3 long isoform to PKCs since it is located near the LIM domain. Our current data demonstrated that the exon 11-positive isoform had only about 50% of the affinity for PKCα and PKCβ compared to the exon 11-negative isoform, while no significant difference was observed in terms of PKCε affinity.

In DM1, PKCs are hyperactivated by unknown mechanisms, leading to stabilization of CUG-BP1 (Kuyumcu-Martinez et al., 2007; Wang et al., 2009). Consistent with this, CUG-BP1 is upregulated in DM1 and is thought to contribute to various phenotypes observed in patients with DM1 (Koshelev et al., 2010; Wang et al., 2009). The importance of PKC activity in DM1 pathogenesis is clear. Thus, LDB3 may function as an anchoring protein to stabilize PKCs to the Z-line and subsequently suppress PKC activation. However, in DM1, the exon 11-positive isoform of LDB3 may have reduced ability to anchor PKC enzymes, thereby promoting hyperactivation of PKCα and PKCβ. Interestingly, PKC activation is achieved rapidly (within 6 h) after administration of CTG repeat RNA (Kuyumcu-Martinez et al., 2007). Thus, hyperactivation of PKC enzymes may be induced through another mechanism.

The LDB3 long isoform also has important functions as a cytoskeletal muscle protein. Mutations in LDB3 have been shown to be associated with dilated cardiomyopathy and left ventricular noncompaction (Arimura et al., 2004, 2009; Cheng et al., 2011; Griggs et al., 2007; Vatta et al., 2003; Xing et al., 2006; Zheng et al., 2009; Zhou et al., 2001). In particular, mutations in the LIM domain of the long isoform have been shown to alter the binding affinity of LDB3 for PKCα, PKCβ, and PKCε (Arimura et al., 2004).

The long isoform has also been suggested to have more important functions than the short isoform. For example, in mice, depletion of a selective cypher (human LDB3) long, not short, isoform has been shown to

lead to late-onset cardiomyopathy (Cheng et al., 2011). In this study, we proved that the long isoforms are predominant ones in DM1 (Fig. 2B and C). Although this difference was statistically significant, the effect might be driven by a small amount of long isoform in FSH and a large amount of long isoform in DM1 (4 and 5). The long isoforms may play an important role in DM1 patients compared to other diseases. Thus, since point mutations in LDB3 have been shown to cause cardiomyopathy, exon inclusion likely has a major impact on muscular phenotype.

In summary, we identified abnormal inclusion of *LDB3* exon 11 specific to DM1 at the RNA and protein level. This inclusion changed the affinity of LDB3 for PKC, indicating that exon 11 may contribute to the activation of PKC in DM1.

Acknowledgments

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