이학석사학위논문

근육 유지에 관여하는 E3 유비퀴틴 라이게이스인 Mind Bomb-1의 기능 연구

The role of E3 ubiquitin ligase

Mind Bomb-1 in muscle maintenance

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I. ABSTRACT

The role of E3 ubiquitin ligase Mind Bomb-1 in muscle maintenance

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Maintenance of skeletal muscle throughout life is essential for quality of life. Hence, it is of great interest to determine the muscle-specific factor responsible to maintain skeletal muscle throughout the lifetime. In skeletal muscle, several muscle-specific E3 ubiquitin ligases are known to play fundamental roles in maintenance of muscle, regulation of muscle protein catabolism and mediation of muscle atrophy. Among the great diversity of E3 ubiquitin ligases, however, there are no studies in muscle on the E3 ubiquitin ligase Mind bomb 1 (Mib1).

In this study, I used Cre-Lox targeted approach to establish a myofiber-specific *Mib1* knockout mice to determine whether myofiber-specific *Mib1* deletion compromises muscle maintenance contributing to progressive muscle atrophy. Here, I first confirmed that the genetic lack of myofiber-specific *Mib1* leads to loss of

muscle mass, decreased cross-sectional area and impaired muscle functions in adult mice. These findings suggest Mib1 may have potential role to prevent skeletal muscle atrophy.

Keywords : Skeletal muscle, Mib1 (Mind Bomb 1), Muscle maintenance, E3 ubiquitin ligase, Muscle atrophy

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III. INTRODUCTION

Skeletal muscle, which comprises a large percentage of body weight, plays important roles for locomotion, structural support, energy production and regulation of body metabolism. Consequently, the maintenance of skeletal muscle is critical for quality of life, and essential for health and even survival (Bonaldo and Sandri, 2013). Unlike postnatal muscle where incorporation of satellite cells into growing fibers largely takes place (Moss and Leblond, 1971; Schultz, 1996; Yablonka-Reuveni, 2011), in adults, the growth of skeletal muscle mass and muscle fiber size (for example, muscle cross-sectional area) depends on the physiological conditions such as growth factors, hormones, nutrients, cytokines, mechanical stress and physical activity. In general, skeletal muscle mass is an important determinant of physical performance, endurance and strength. A decrease of muscle cross-sectional area with subsequent reduction in whole muscle volume and mass, but no decrease in fiber numbers is the major characteristic of muscle atrophy (Nicks et al., 1989). On the other hand, the age-related muscle atrophy is accompanied by reduction in both cross-sectional area and fiber numbers (Lexell et al., 1988).

Muscle atrophy is a deliberating consequences of aging, denervation (for example, in patients with spinal cord injuries or neuromuscular diseases), inactivity (for example, during prolonged bed rest or cast-immobilization) and systemic response to fasting and various chronic diseases (Bodine and Baehr, 2014; Brooks

and Myburgh, 2014; Cohen et al., 2015). The process of muscle atrophy is tightly controlled by many signaling pathways and results in changes of balance between protein breakdown and synthesis. Amongst cellular and molecular pathways regulating skeletal muscle atrophy, the ubiquitin proteasome system is responsible for depletion of most proteins in atrophying skeletal muscles (Brooks and Myburgh, 2014; Costelli and Baccino, 2003; Solomon and Goldberg, 1996). Once ubiquitin is activated by ubiquitin-activating enzyme (E1), it is transferred to active site of ubiquitin-conjugating enzyme (E2). Subsequently, activated E3 ubiquitin ligase recognizes the substrates and adds ubiquitin moieties to target proteins, thereby triggering degradation of ubiquitin-conjugated substrates (Lyon et al., 2013; Weissman et al., 2011). Polyubiquitinated proteins are mainly degraded by 26S proteasome, while monoubiquitinated proteins are degraded by lysosomal cathepsins (Fanzani et al., 2012; Marmor and Yarden, 2004). Consequently, activation of E3 ubiquitin ligases in skeletal muscles during muscle atrophy results in the targeting and degradation of substrates involved in diverse cellular processes.

The activity of most E3 ubiquitin ligases is specified by C-terminal RING domains, which are implicated in proteasome-dependent proteolysis, receptor endocytosis, and vesicular trafficking (Deshaies and Joazeiro, 2009). Several E3 ubiquitin ligases have been reported to implicate in muscle development, maintenance, and/or atrophy, including MuRF1 (Bodine et al., 2001), MAFbx/Atrogin-1 (Bodine et al., 2001; Gomes et al., 2001), TRIM32 (Cohen et al.,

2012) and TRAF6 (Paul et al., 2010). MuRF1 and TRAF6, which contain a RING finger domain, are known to target several myofibrillar and structural proteins (Paul et al., 2010; Witt et al., 2005). Similarly, MAFbx/Atrogin-1, which contains cullin-RING finger domain, targets sarcomeric proteins, myogenic regulatory factors and eukaryotic initiation factors (Csibi et al., 2009; Tintignac et al., 2005). Unlike MuRF1 and MAFbx/Atrogin-1 that are muscle specific, TRIM32, which ubiquitinates desmin cytoskeleton and myofibrils (Cohen et al., 2012), is expressed ubiquitously with 100-fold higher expression levels in brain than in skeletal muscles (Kudryashova et al., 2009). However, interestingly, mutations in TRIM32 results in mild dystrophy, limb gridle muscular dystrophy type 2H, and sarcotubular myopathy (Kudryashova et al., 2009; Schoser et al., 2005) and loss of TRIM 32 in mice results in myopathy and neurological defects (Kudryashova et al., 2012; Kudryashova et al., 2009).

Mind bomb 1 (Mib1) ubiquitin ligase is essential for cytoplasmic ubiquitin-mediated endocytosis of Notch ligands (Koo et al., 2007). Mib1 contains two mib/herc2 domains, one zz zinc finger domain, two Mib repeats, eight Ankyrin repeat domains and three RING finger domains (Itoh et al., 2003; Koo et al., 2005; McMillan et al., 2015). Similar to TRIM32, Mib1 ubiquitously expressed in embryo and adult tissues (Burns et al., 2005; Haddon et al., 1998; Itoh et al., 2003; Koo et al., 2005; Lawson et al., 2001; Luxan et al., 2013). Recently, Tseng et al. reported putative Mib1-binding proteins via yeast-two-hybrid screening and showed that several Mib1-interacting proteins involved in cytoskeleton members such as myosin light

chain, actinin alpha 2-4, cofilin 1, dystobrevin, and dystrophin-associated protein A1 (Tseng et al., 2014). Considering diverse range of functions of E3 ubiquitin ligases, it is likely that there are more unknown Mib1-interacting proteins participating in the development or maintenance of skeletal muscle. However, the role of Mib1 in skeletal muscle has not been investigated.

Through the myofiber-specific Mib1 knockout mice, I investigated the role of Mib1 in adult muscle maintenance. In this study, I found that the ablation of Mib1 in myofibers causes loss of muscle mass and reduction in cross-sectional area, and impaired muscle functions in adulthood. Collectively, my results raise the possibility that there are unknown Mib1-binding substrates which plays important role in adult muscle maintenance.

IV. MATERIALS AND METHODS

IV-1. Mice

Mice with a *Mib1* gene (Mib1$^{f/f}$) flanked by a pair of *loxP* sites were previously (Koo et al., 2007). Myofiber-specific transgenic mice expressing Cre recombinase under control of the *MCK* promoter (Bruning et al., 1998) were purchased from The Jackson Laboratory (Bar Harbor, Maine, USA). For MF-specific deletion studies, *Mib1*$^{f/f}$ mice were crossed with *MCK-Cre* mice to generate *MCK-Cre;Mib1*$^{f/f}$ mice. Young (3 months of age), adult (6-9 months of age) and middle-aged (16 months of age) male *MCK-Cre;Mib1*$^{f/f}$ mice and littermate control mice were used for experiments. All of these mouse lines were backcrossed onto a C57BL/6 background and were housed and handled according to the guidelines of the ethical committees at Seoul National University.

IV-2. Grip Strength

Whole-limb grip strength was assessed by allowing mice to grab a grid which is attached to a grip strength test meter (Grip strength test, Bioseb, France). The mouse is pulled horizontally by the tail away from the grid and the peak force was measured (Bonetto et al., 2015). The test was performed five times with 5 seconds of recovery. The maximum absolute grip strength (g force; in grams) for each mouse

was normalized to body mass (g) to determine each grip strength for each mouse. All experiments were performed in a blind fashion.

IV-3. Whole-Limb Hanging Test

Whole-limb hanging test was assessed by allowing mice to grab a wire mesh (1 cm X 1 cm) while carrying a weight (8-10 % of their body) attached to their tails. Each mouse was placed on a wire mesh, which was inverted and suspended above soft bedding to protect mice from falling off the wire mesh. The latency of mice to fall off was recorded three times with a rest interval between each trial of about 10 min (Bonetto et al., 2015; Luk et al., 2012). The maximum hang time for each mouse was used. All experiments were performed in a blind fashion.

IV-4. Treadmill Exercise Test

Untrained mice were evaluated for their running capacity. The mice were familiarized with the treadmill for 3 days before the test by running 10 min per day at 10 m/min. Mice were then subjected to a high intensity running regime with 5 m/min speed increments every 5 minutes up to 15 m/min speed until exhaustion. Running time and distance were recorded and collected for each mouse. Mice were sacrificed one week after exercise tests.

IV-5. Histological Analysis

Hindlimb muscles were dissected, weighed, immersed in PBS and embedded in Tissue Tek OCT compound (Sakura Fineteck, Toerrance, CA). Dissected muscle were quickly frozen in liquid nitrogen and stored at -80 °C prior to sectioning. Muscles were sectioned on a cryostat at 7-μm and used for histochemistry or immunohistochemistry. For histological analysis, muscle sections were fixed in 4% paraformaldehyde overnight at 4°C and stained with hematoxylin and eosin (H&E).

IV-6. Immunohistochemical Analysis

For immunohistochemistry, muscle sections were fixed with 4% paraformaldehyde in PBS for 10 min, washed in PBS, permeabilized with permeabilization solution (0.2% Tween-20 in 5% BSA in PBS) 10 min and treated with MOM blocking solution for 1 h at room temperature, according to the manufacturer's instructions (FMK-2201; Vector Laboratories, Burlingame, CA, USA). The sections were then incubated with rat anti-laminin (1:2,000, Abcam), mouse anti-MyHC1 (1:300, DSHB) and mouse anti-MyHC2a (1:300, DSHB) at 4°C overnight. The slides were washed with PBS several times and incubated with secondary antibodies for 1 h at room temperature. The slides were mounted with Vectashield (H-1001; Vector Laboratories, Burlingame, CA, USA) after washing with PBS. Images were visualized with an Observer Z1 fluorescent microscope (Zeiss) and captured with a Spot Flex camera. For fiber cross-sectional area calculation, Leopard (ZOOTOS,

Korea) was used.

IV-7. Immunoblotting Analysis

For immunoblotting, *gastrocnemius* muscles were homogenized in 500mL RIPA buffer (50 mM Tris-HCl, pH 7.5, 0.5% SDS, 20 µg/mL aprotinin, 20 µg/mL leupeptin, 10 µg/mL phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, 10 mM sodium pyrophosphate, 10 mM sodium fluoride, and 1 mM dithiothreitol). Bradford's reagent (Bio-Rad Laboratories, Hercules, CA, USA) was used for measuring total protein concentrations. Muscle extracts were separated by electrophoresis in 6-10% polyacrylamide gels and transferred to PVDF membranes (Milipore, Overijse, Belgium). The membranes were incubated with antibodies to Mib1 (Abfrontier, 1:200), MyHC1 (DSHB, 1:500), Ubiquitin (Santa Cruz, 1:400), and tubulin (Abcam, 1:7,000) overnight at 4°C or 2 h at room temperature followed by corresponding secondary antibodies (Sigma-Aldrich or Invitrogen). Protein bands were detected with enhanced chemiluminescence (Amersham Pharmacia Biotech) using LAS imaging system (Fujifilm, Tokyo, Japan). β-tubulin was used as loading control.

IV-8. qRT-PCR Analysis

Total RNA was extracted from the freshly isolated GA muscles using TRI Reagent (Sigma-Aldrich) and analyzed by qRT-PCR. The complementary DNA synthesis was

performed according to the manufacturer's instructions (Omniscript kit; Qiagen). The data were normalized to β-actin housekeeping genes. Primer sequences for qRT-PCR are as follows: Mib1, forward, 5'-TCCTGGACTGAACCTGCTCT-3', and reverse, 5'- AGTGGGTTCTCGGAGTCCTT-3', LC3, forward, 5'-CATGAGCGAGTTGGTCAAGA-3', and reverse, 5'-CCATGCTGTGCTGGTTGA-3', and Bnip3, forward, 5'- CCTGTCGCAGTTGGGTTC-3', and reverse, 5'-GAAGTGCAGTTCTACCCAGGAG-3', and b-actin, forward, 5'-TCATGAAGTGTGACGTTGAC-3', and reverse, 5'-CCTAGAAGCATTTGCGGTGC-3'.

IV-9. Statistical Analysis

Statistical significance was determined by applying student *t*-test to raw values from at least 3 independent experiments. All the error bars represent the S.E.M. A p value of < 0.05 considered statistically significant at the 95% confidence level.

V. RESULTS

V-1. Myofiber-specific deletion of *Mib1* results in muscle atrophy in adulthood

To investigate whether loss of Mib1 in muscle has adverse effects on the maintenance of skeletal muscle, I crossed *muscle creatine kinase (MCK)-Cre* transgenic mice (Bruning et al., 1998) with *Mib1*$^{f/f}$ mice (Koo et al., 2007) to generate myofiber-specific *Mib1* gene-deleted mice (*MCK-Cre;Mib1*$^{f/f}$; hereafter Mib1 cKO). Since Mib1 plays a significant role in Notch signaling (Itoh et al., 2003; Koo et al., 2005) that is necessary to maintain muscle stem cells during skeletal muscle development (Bjornson et al., 2012; Mourikis et al., 2012; Mourikis and Tajbakhsh, 2014), I investigated whether Mib1 cKO mice have normal skeletal muscle development. We first examined the longitudinal changes in body mass of Mib1 cKO and wild-type (WT) control mice between 1 and 17 months of age. Mib1 cKO and WT mice, which fed ad libitum, showed a similar increase of body weight during postnatal development (Figure 1A). To determine the changes in muscle morphology with age, I analyzed hindlimb muscles [*tibialis anterior* (TA), *gastrocnemius* (GA), and *quadriceps femoris* (Q) muscles] of young (3 months of age), adult (6-9 months of age) and middle-aged (MA; 16 months of age) male mice.

Muscle weight in young Mib1 cKO mice was similar to that of WT mice, suggesting Mib1-deficient skeletal muscles develop normally during postnatal growth. However, MA Mib1 cKO mice showed significant decline in the weight of GA and Q muscles (Figure 1C), although the Q muscles did not quite reach statistical significance (p=0.057). Consistently, histological analysis of GA muscles was apparently normal in young Mib1 cKO mice whereas showed narrow myofibers in adult and MA Mib1 cKO mice compared to that of WT mice, indicating age-associated gradual loss of skeletal muscle in Mib1 cKO mice.

Since reduction in muscle volume and mass is accompanied by decreased muscle fiber cross sectional area (CSA) and/or number of fibers (Glass, 2003; McKinnell and Rudnicki, 2004; Romanick et al., 2013), I examined the impact of loss of Mib1 in myofiber on mean CSA of muscles and total muscle fiber numbers. On immunohistochemical examination of TA muscles of Mib1 cKO and WT mice, the number of muscle fibers of adult and MA TA muscles was comparable between groups (Figure 1G). However, there were significant decreases in CSA of adult (Figure 1F and H) and MA Mib1 cKO TA muscles (Data not shown), suggesting the reduction in muscle results from decrease in muscle CSA, but not the decline in muscle fiber numbers.

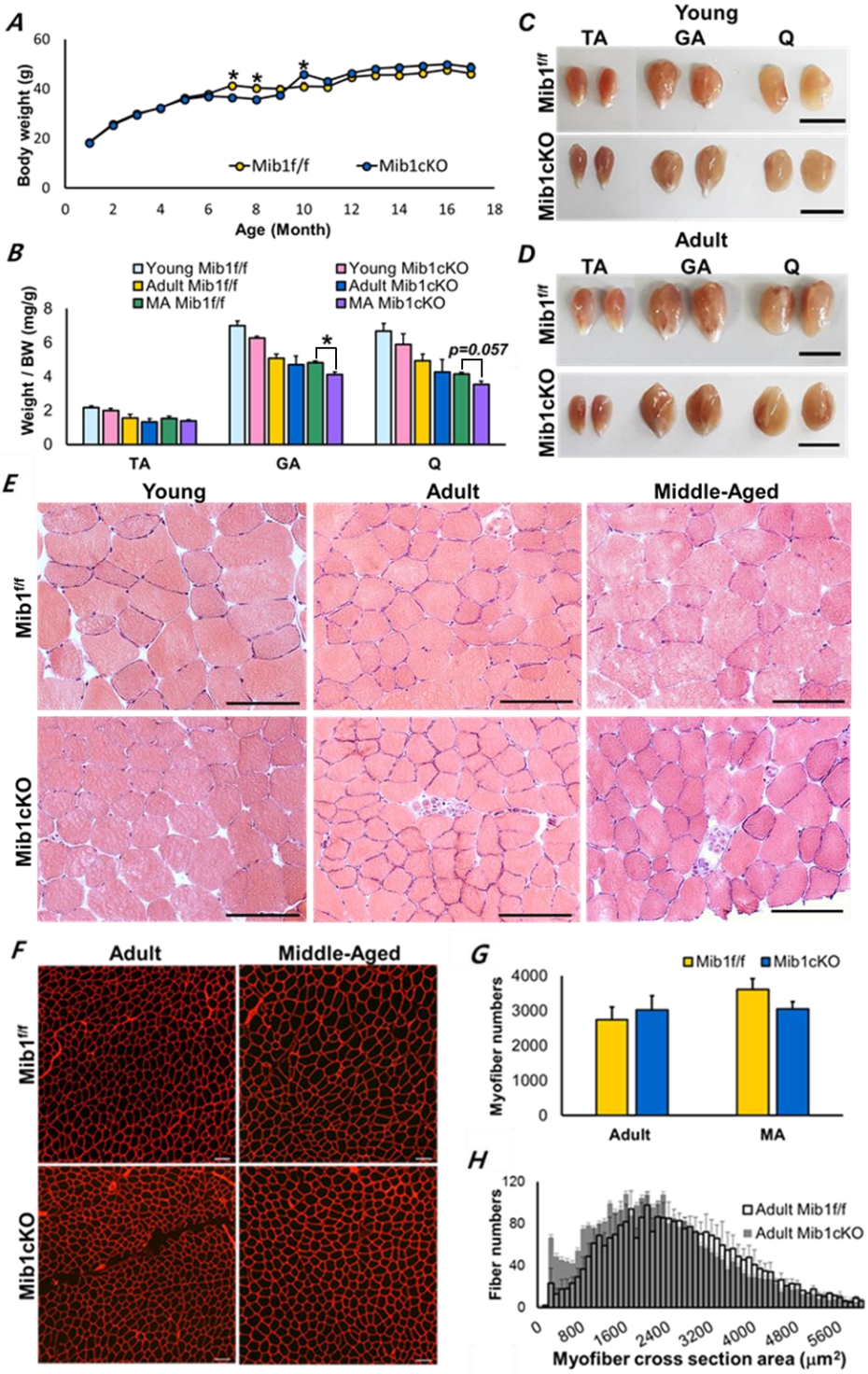
A
Body weight (g)
Age (Month)
Mib1f/f
Mib1cKO
B
Weight / BW (mg/g)
Young Mib1f/f
Adult Mib1f/f
MA Mib1f/f
Young Mib1cKO
Adult Mib1cKO
MA Mib1cKO
p=0.057
TA
GA
Q
C
Young
TA
GA
Q
Mib1f/f
Mib1cKO
D
Adult
TA
GA
Q
Mib1f/f
Mib1cKO
E
Young
Adult
Middle-Aged
Mib1f/f
Mib1cKO
F
Adult
Middle-Aged
Mib1f/f
Mib1cKO
G
Mib1f/f
Mib1cKO
Myofiber numbers
Adult
MA
H
Fiber numbers
Adult Mib1f/f
Adult Mib1cKO
Myofiber cross section area (μm²)

Figure 1. Ablation of Mib1 in myofiber induces muscle loss in mice.

(A) Body weight curves of male WT and Mib1 cKO mice fed ad libitum. (B) Relative hindlimb muscle [*tibialis anterior* (TA), *gastrocnemius* (GA) and *quadriceps femoris* (Q) muscles] divided by body weight at indicated ages. (C-D) Optic images of hindlimb muscles of young (3 months of age, C) and adult (9 months of age, D) WT and Mib1 cKO mice. (E) H&E stained images of TA muscles of young, adult and middle-aged (16 months of age) WT and Mib1 cKO mice. (F) Laminin-stained TA muscles of adult and MA WT and Mib1 cKO mice. (G) Myofiber numbers of TA muscles of adult and MA WT and Mib1 cKO mice. (H) Morphometric quantification of cross-sectional area in Laminin-stained TA muscle of adult and MA WT and Mib1 cKO mice. Young, adult and MA (middle-aged) represent 3-, 6 to 9-, and 16 months of age, respectively. Scale bars, 1 cm (C, D) and 100 μm (E, F). Data are mean ± SEM. N = 3-4 mice per each genotype and age. *$p < 0.05$.

V-2. Ablation of *Mib1* in myofiber leads to impaired muscle function

To examine whether the ablation of *Mib1* led to changes in muscle function, I performed several muscle function tests such as treadmill exercise, grip strength test and hanging test. For treadmill exercise tests, mice were subjected to a high intensity running regime with 5 m/min speed increments every 5 minutes up to 15 m/min speed until exhaustion (Figure 2A). Both adult and MA Mib1 cKO mice showed lower endurance run capacity than age-matched WT mice (Figure 2B-C). Additionally, I analyzed the muscle strength to evaluate neuromuscular performance (Bonetto et al., 2015). There was no difference in grip strength (Figure 2D-E), suggesting that loss of Mib1 has no adverse effects on muscle strength. However, when I further performed four limb-hanging test (Balkaya et al., 2013; Bonetto et al., 2015) to assure normal neuromuscular function in Mib1 cKO mice, Mib1 cKO mice showed poor neuromuscular performance (Figure 2F-G). Unfortunately, some mice seem to avoid hanging by falling off it on purpose albeit mice were adapted prior to tests. Hence, larger number of mice are required to validate neuromuscular functions of Mib1 cKO mice. Taken together, these data indicate that the loss of Mib1 in myofibers result in lower running capacity, and mild or no gross impairment of motor functions at adulthood.

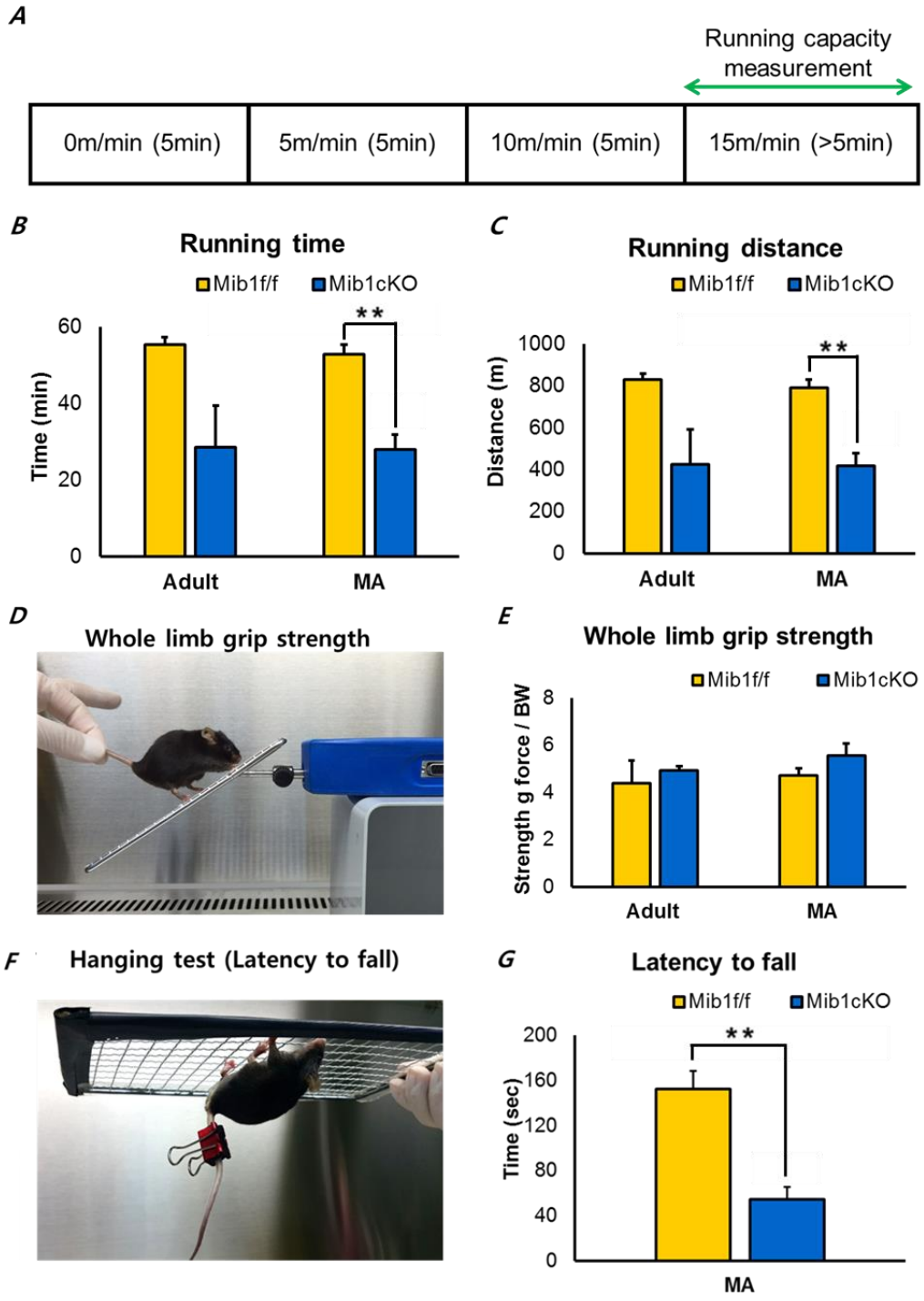
A
Running capacity measurement
0m/min (5min)
5m/min (5min)
10m/min (5min)
15m/min (>5min)
B
Running time
Mib1f/f
Mib1cKO
Time (min)
60
40
20
0
**
Adult
MA
C
Running distance
Mib1f/f
Mib1cKO
Distance (m)
1000
800
600
400
200
0
**
Adult
MA
D
Whole limb grip strength
E
Whole limb grip strength
Mib1f/f
Mib1cKO
Strength g force / BW
8
6
4
2
0
Adult
MA
F
Hanging test (Latency to fall)
G
Latency to fall
Mib1f/f
Mib1cKO
Time (sec)
200
160
120
80
40
0
**
MA

Figure 2. Myofiber-specific *Mib1* ablation leads to impaired muscle function.

(A) Experimental design illustrating the treadmill exercise paradigm. Mice were familiarized to treadmill running. The initial speed of 5 m/min was increased by 5 m/min every 5 min. The running capacity was measured at maximum speed of 15 m/min until exhaustion. (B-C) Running time (B) and running distance (C) of WT and Mib1 cKO mice at indicated ages. (D) Representative images of whole-limb grip strength. (E) Whole-limb grip strength of WT and Mib1 cKO mice at indicated ages. Maximum absolute grip strength (g force; in grams) for each mouse was normalized to body mass (g). (F) Representative images of hanging test. (G) Hanging wire tests of WT and Mib1 cKO mice at indicated ages. Adult and MA (middle-aged) represent 6 to 9-, and 16 months of age, respectively. Data are mean ± SEM. N = 3-4 mice per each genotype and age. **$p < 0.01$.

V-3. Mib1 is required for the activation of the ubiquitin-mediated proteolysis

The ubiquitin-mediated proteolysis is the major pathway which causes the degradation of muscle proteins in atrophying conditions (Brooks and Myburgh, 2014; Costelli and Baccino, 2003; Solomon and Goldberg, 1996). I investigated the possibility of whether Mib1 is involved in ubiquitin-mediated protein degradation pathway during muscle atrophy. GA muscles were isolated from adult and MA WT and Mib1 cKO mice, and used to measure the levels of ubiquitinated proteins by immunoblotting. Interestingly, the protein ubiquitylation was considerably lower in GA muscles of Mib1 cKO mice than in those of WT mice (Figure 3A). Moreover, decreased levels of specific bands in MA Mi1 cKO muscles were observed (Figure 3A, red box), indicating that Mib1 is involved in the degradation of muscle proteins. During muscle atrophy, several contractile proteins such as myosin heavy chain, one of most abundant protein in skeletal muscle, undergo degradation process (Cohen et al., 2009; Cohen et al., 2015). To determine that Mib1 is involved in degradation of muscle proteins, immunoblotting on myosin heavy chain fast type (MyHC1) protein, which is abundantly expressed in GA muscles (Sher and Cardasis, 1976), was performed. There was no significant degradation of MyHC1 in GA muscles from MA Mib1 cKO mice compared to that of WT mice (Figure 3A). Consistently, immunohistochemical analysis of myosin heavy chain fast type

(MyHC2a) and myosin heavy chain slow type (MyHC1) expression in TA muscles revealed the similar distributions of myosin heavy chain in Mib1 cKO and WT mice (Figure 3B-D). In addition, the distributions of myosin heavy chain in GA muscles from Mib1 cKO mice was comparable to that of WT mice (Data not shown). Taken together, these results suggest that Mib1 functions through the activation of ubiquitin-proteasome pathway but did not trigger the degradation of myosin heavy chains.

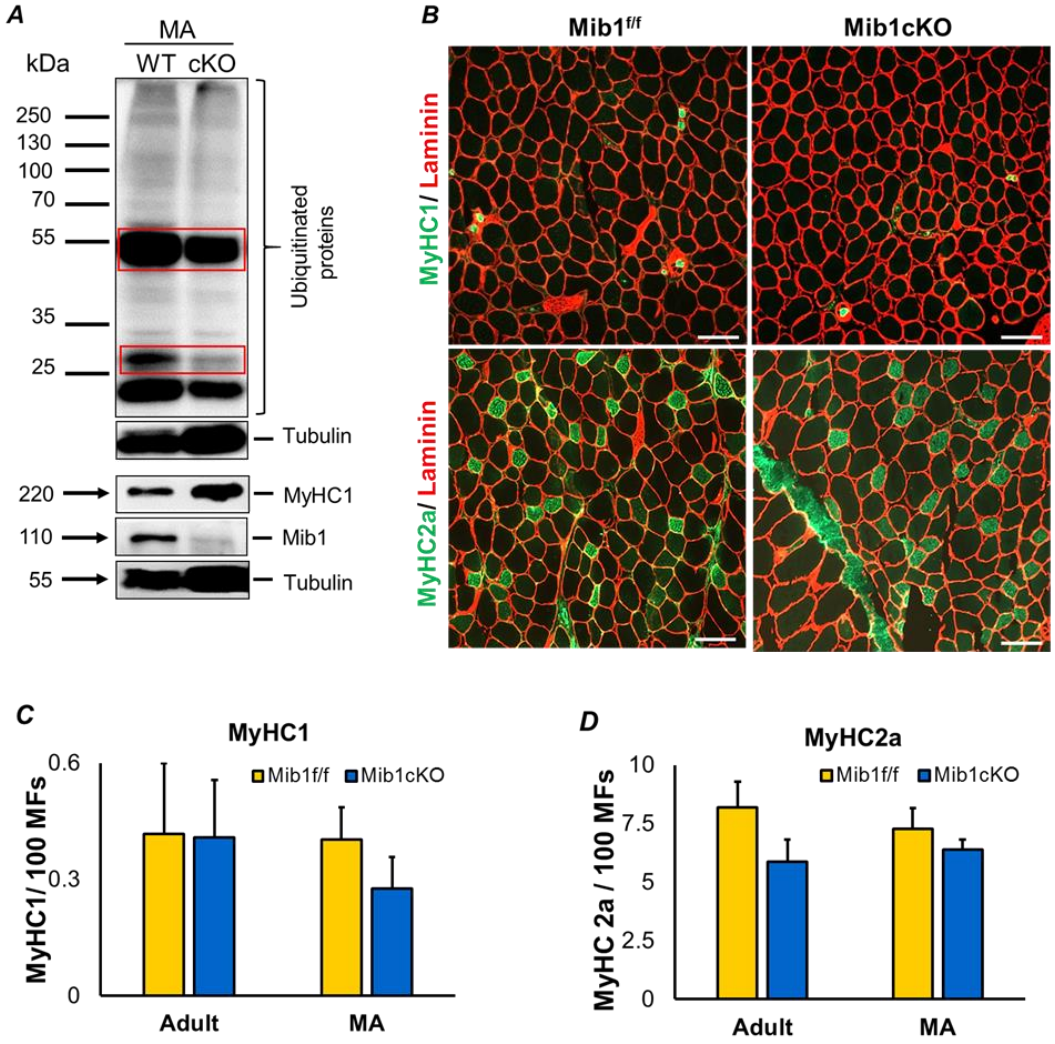
A
MA
kDa
WT cKO
250
130
100
70
55
35
25
Ubiquitinated proteins
Tubulin
220
MyHC1
110
Mib1
55
Tubulin
B
Mib1f/f
Mib1cKO
MyHC1/ Laminin
MyHC2a/ Laminin
C
MyHC1
Mib1f/f
Mib1cKO
MyHC1/ 100 MFs
0.6
0.3
0
Adult
MA
D
MyHC2a
Mib1f/f
Mib1cKO
MyHC 2a / 100 MFs
10
7.5
5
2.5
0
Adult
MA

Figure 3. Myofiber-specific Mib1 regulates ubiquitin-mediated proteolysis.

(A) Representative immunoblots for ubiquitin, MyHC1, and Mib1 in GA muscles of WT and Mib1 cKO mice at indicated ages. Tubulin was used as loading control. Red box indicates the decrease in expression level of ubiquitin in Mib1 cKO mice compared to that of WT mice. (B) Representative images of Laminin and MyHC1/2a-stained TA muscles of adult WT and Mib1 cKO mice. (C-D) Quantification of myosin heavy chain slow type (MyHC1) and myosin heavy chain fast type (MyHC2a) in TA muscles of WT and Mib1 cKO mice at middle-aged. Adult and MA (middle-aged) represent 6 to 9-, and 16 months of age, respectively. Data are mean ± SEM. N = 3-4 mice per each genotype and age. *$p < 0.05$.

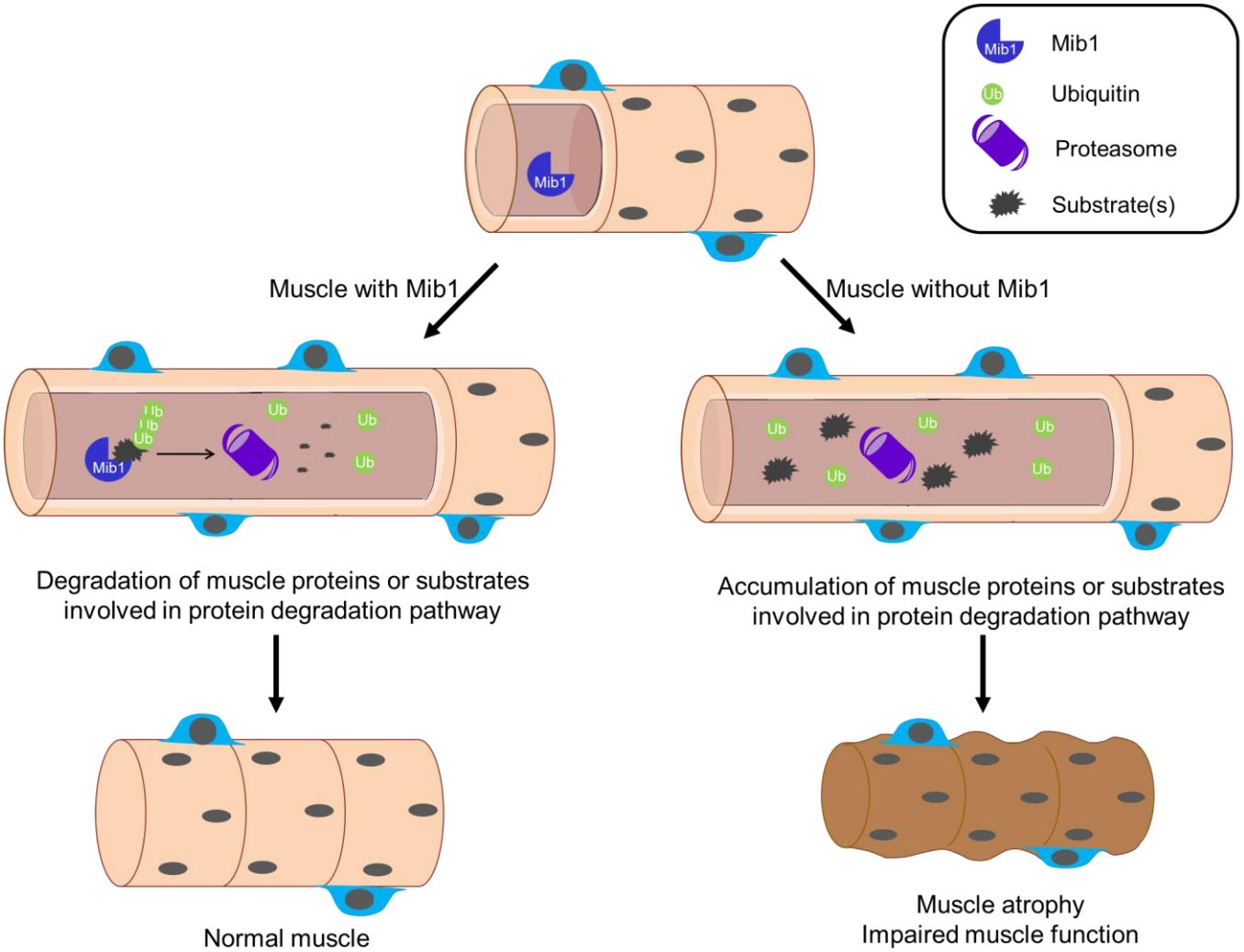
Mib1
Ubiquitin
Proteasome
Substrate(s)
Mib1
Muscle with Mib1
Muscle without Mib1
Mib1
Ub
Ub
Ub
Ub
Ub
Ub
Ub
Ub
Ub
Degradation of muscle proteins or substrates
involved in protein degradation pathway
Accumulation of muscle proteins or substrates
involved in protein degradation pathway
Normal muscle
Muscle atrophy
Impaired muscle function

Figure 4. A schematic depiction of Mib1 regulating muscle maintenance and function

During normal physiological condition, the protein homeostasis is regulated by several E3 ubiquitin ligases including Mind Bomb 1 (Mib1) E3 ubiquitin ligases. In that, Mib1-mediated ubiquitin-proteasome system degrades the muscle proteins or substrates involved in protein degradation pathway, thereby maintaining muscle homeostasis. However, in the absence of Mib1 in myofiber, these muscle proteins or substrates accumulate leading to generation of harsh conditions associated with muscle atrophy and impaired muscle function.

VI. DISCUSSION

In this study, I investigated the effects of loss of Mib1 in muscle maintenance. This finding indicates that ablation of *Mib1* is detrimental to the muscle maintenance and muscle adaptation to endurance aerobic exercise but may not have adverse impact on motor coordination. The loss of muscle mass associated with reduction in muscle cross-sectional area and decreased muscle function in Mib1 cKO mice clearly mimic the clinical manifestations of muscle atrophy (Berg et al., 1991; Dodson et al., 2011; Gogia et al., 1988; Larsson et al., 1979; MacDougall et al., 1977). The most surprising result of my study was that the loss of Mib1 in myofiber indeed caused a decrease in the level of ubiquitinated proteins (Figure 3A). Given that, I can reasonably expect that the role of Mib1 in muscle would be the degradation of degenerating muscle proteins (Figure 4) like parkin, which is an E3 ubiquitin ligase participating in the regulation of proteasomal degradation of abnormal mitochondria (Castillo-Quan, 2011).

Several studies reveal the significant role of ubiquitin-mediated pathway in muscle atrophy (Cohen et al., 2015; Lyon et al., 2013; Weissman et al., 2011). In order to determine the molecular mechanism of Mib1 in ubiquitin-mediated proteolysis in muscle atrophy, the further studies on Mib1 binding substrate and its potential function in muscle maintenance should be conducted. I am currently

planning to perform yeast-two-hybrid screening on single myofiber and immunoprecipitation experiment using LC-MS/MS to expand the Mib1 signaling network in muscle maintenance. Although recent studies reported the list of potential Mib1 binding partners from adult rat brain and zebrafish (Mertz et al., 2015; Tseng et al., 2014), the elaborated screening on single muscle fibers are required to determine the novel interaction partners of Mib1 which play significant role solely in myofibers. My further works on Mib1 will expand the current understanding on Mib1.

In human, *MIB1* gene is located on the long arm of chromosome 18q 11.2 (Wystub et al., 2013). According to clinical report, diseases associated with deletion of long arm of chromosome 18 are characterized by low muscle tone, hypotonia (Cody et al., 2007; Strathdee et al., 1995; Surh et al., 1991; Wertelecki and Gerald, 1971). Unlike healthy muscle which never fully relaxed, hypotonia is a symptom of low muscular tone associated with decreased tension and stiffness of muscles to stretching which is caused by systemic diseases and diseases of the nervous system (Leyenaar et al., 2005; Lisi and Cohn, 2011). Furthermore, the genetic conditions such as Down Syndrome, Prader-Willi syndrome, Tay-Sachs disease, spinal muscular atrophy, Charcot-Marie-Tooth disease and muscular dystrophy are known to cause hypotonia. Although several types of therapy for hypotonia have been performed, currently, there are no direct treatment for hypotonia (Leyenaar et al., 2005; Lisi and Cohn, 2011). In that, unraveling the function of Mib1 and

interacting substrates in muscle maintenance would suggest a therapeutic approach to the treatment of hypotonia and muscle atrophy.

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VIII. 요약 (국문초록)

사람이 삶의 질을 향상시키고 건강한 신체를 갖추기 위해서는 근육의 유지가 무엇보다도 중요하다. 따라서 일생동안 근육을 유지시킬 수 있는 근육 특이적인 인자를 찾기 위해 연구자들이 많은 관심을 갖고 연구를 진행하고 있다. 근육에는 근육의 유지, 근육 단밸직의 의화작용, 근육위축증을 조절하는 여러 E3 유비퀴틴 라이게이즈들이 존재하고 있다고 알려져있으나, 아직까지 E3 유비퀴틴 라이게이즈인 Mind Bomb 1 (Mib1)이 근육에서 어떠한 기능을 하는지에 대해 정확하게 규명되어 있지 않다. 본 연구에서는, Cre-Lox 시스템을 이용해 만든 근육섬유 특이적 Mib1 적중 마우스를 분석함으로써 Mib1이 결손된 경우 근육의 무게와 근육섬유 단면적이 감소되고, 근육의 기능이 현저하게 저해되는 것을 발견하였다. 또한, 근육섬유 특이적 Mib1 적중 마우스의 근육에서 유비퀴틴화가 감소된 것을 확인하였고, 이는 Mib1이 유비퀴틴 프로테아좀 기전을 통해 근육을 유지한다는 것을 증명해주고 있다. 이러한 연구 결과는 근육섬유에 존재하는 Mib1이 유비퀴틴 프로테아좀 시스템을 통해 손상된 근육 단백질들을 유비퀴틴화 시켜 분해시키며, 이러한 기능이 저해될 경우 근육 위축증이 일어나고 근육기능이 소실될 수 있다는 사실을 증명해주고 있다.

주요어 : 근육, Mib1 (Mind Bomb 1), 근육 유지, 근육 위축증, E3 유비퀴틴 라이게이즈

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