수의학 석사 학위논문

Effects of adipose tissue-derived miRNA on skeletal muscle development and regeneration

지방 조직 유래 miRNA가 근육의 발달 및 재생에 미치는 영향

2022년 2월

서울대학교 대학원

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이 논문을 수의학 석사 학위논문으로 제출함
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Abstract

Adipose tissue is a major site for energy storage and plays a key role in the regulation of metabolism through the release of miRNA. It is well known about the effects of adipose tissue-derived miRNA on adipose tissue itself. However, they circulate and allow distant organs to communicate with each other. It has not been studied well about the effects of miRNA from adipose tissue on other tissues. Here we showed the effects on skeletal muscle with mice, an adipose tissue-specific knockout of the microRNA-processing enzyme Dicer (ADicer-KO). There were significant differences in skeletal muscles between WT and ADicer-KO mice in 9 weeks old, not in postnatal 10 days. ADicer-KO mice had smaller skeletal muscles and decreased expression level of myogenesis-related genes in TA muscle. Also, ADicer-KO mice showed lower expression levels of myogenic regulatory factors followed by deficient regeneration capacity after chemical-induced skeletal muscle injury. Thus, these findings indicated that adipose tissue-derived miRNA can regulate the development and regeneration of skeletal muscle.

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1. Introduction

White adipose tissue (WAT) is mainly located in subcutaneous and intra-abdominal regions and is the major site of energy storage. On the other hand, Brown adipose tissue (BAT) is important for producing heat by expending energy [1, 2]. Adipose tissue dysfunction is determined by an impaired adipocyte hypertrophy, altered lipid metabolism, and local inflammation [3, 4]. Changes of body fat distribution can change whole body metabolism [5, 6].

Dicer is a member of the ribonuclease (RNase) III family. It is well known as the endonuclease that functions in the RNA interference (RNAi) pathway to cleave long double-stranded RNA (dsRNA) molecules into short dsRNA molecules, including microRNA (miRNA) and small interfering RNA (siRNA) [7, 8].

MicroRNAs (miRNAs) are non-coding RNAs of 19-22 nucleotides and participate in post-transcriptional regulation. Many miRNAs exist in the circulation, a large fraction of which are in exosomes [9]. It is well known about the effect of miRNA from adipose tissue on adipose tissue itself [10, 11]. In preadipocytes, expression of miR-103 or miR-143 accelerated adipogenesis [12]. miR-193b-365 was an important regulator for brown fat

differentiation and miR-196a induced functional brown adipocytes in WAT through the suppression of Hoxc8 [13, 14].

Skeletal muscle development is regulated by the expression of myogenic regulatory factors (MRFs). They are a family of skeletal muscle-specific, basic helix-loop-helix transcription factors, including MyoD, Myf5, Myogenin and Myogenic Regulatory Factor4 (MRF4) [15]. When muscle stem cells are activated, they upregulate the expression of MyoD and enter the cell cycle to proliferate as myoblasts. Then, they differentiate by downregulating the expression level of Pax7 and upregulating the levels of Myogenin and MRF4 [16]. Also, regeneration of skeletal muscle after injury is also regulated by MRFs. Mice lacking MyoD and Myf5 have severely impaired regenerative ability with thin myofibers and many mononucleated cells [15, 17]. To study the process of skeletal muscle regeneration, we induced skeletal muscle injury by injecting barium chloride ($BaCl_2$) into tibialis anterior (TA) muscle. The use of $BaCl_2$ is the most reproducible and the easiest way to induce skeletal muscle injury and regeneration. It injured myofibers through depolarization of the sarcolemma, causing Ca^{2+} overload with transient contraction, leading to proteolysis and membrane rupture [18, 19].

Exosomal miRNAs released from adipose tissue are new

players that regulate systemic metabolism by connecting distant organs [20]. Recent study has reported that miRNAs from adipose tissue have effects on other tissues. Adipose tissue-derived miR-99b suppressed FGF-21 expression and improved glucose tolerance in the liver. miR-130b downregulated PGC-1 α expression in the skeletal muscle cell [21]. However, its effects on other organs have not been studied well. In this study, we will discuss adipose tissue-derived miRNA and their roles in muscle development and regeneration. We hypothesized that miRNAs from adipose tissue play a role in muscle development and regulate the expression of myogenic regulatory factors during regeneration.

2. Materials and Methods

Animal

Adipose tissue-specific Dicer-knockout mice (ADicer-KO) were generated by breeding dicer fl/fl mice (B6.Cg-Dicer1^{tm1Bdh}/J) with mice carrying an adiponectin promoter Cre transgene (B6;FVB-Tg(Adipoq-cre)1Evdr/J). Mice were maintained at temperature of (22-24) ℃, humidity of (50-60) %, with a 12 h light/dark cycle. They were in a specific pathogen-free barrier facility and had a free access to a regular chow diet (NIH-31, Ziegler Bros, PA), along with tap water. All animal experimental protocol was performed according to the "Guide for Animal Experiments" (Edited by the Korean Academy of Medical Sciences) and approved by the Institutional Animal Care and Use Committee (IACUC) of Seoul National University (Approval Number SNU-200903-3).

Body composition

Fat and lean body masses were assessed by 1H magnetic resonance spectroscopy in postnatal 10 days and 9 weeks old mice. Body Composition was analyzed by Nuclear Magnetic

Resonance (NMR) methods (Minispec LF-50, Bruker BioSpin, MA).

$BaCl_2$ injection

To induce skeletal muscle injury in vivo, mice were anesthetized with isoflurane and then 1.2% $BaCl_2$ (202738, Sigma) was injected unilaterally into the TA (50 μL). Mice were kept warm during recovery and then returned to their cage. Following tissue harvest, mice were killed by CO_2.

real-time PCR analysis

Total RNA from injured or uninjured TA skeletal muscle was extracted with TRIzol reagent (Life Technologies, USA) according to the manufacturer's standards. First-strand cDNA for PCR analyses was generated with AccuPower® RT PreMix (Bioneer, Korea). Real-time PCR analysis was performed using QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, USA). The qRT-PCR primers were described on Table 1.

Western blot analysis

Fat tissues were lysed in a RIPA Lysis and Extraction Buffer (Thermo, USA) and protease and phosphatase inhibitors (genDEPOT, USA). Proteins were separated by SDS-PAGE on 8 to 10% gradient gels and transferred to a transfer membrane. Membranes were washed 2 × 10 min with TBST (0.247M Tris, 27mM Potassium chloride, 1.37M Sodium chloride, 0.5% Tween-20) and blocked with 5% skim milk based on TBST for 1 hr at room temperature. After 10 min washing, it is incubated with primary antibodies against Dicer (PA5-23088, Invitrogen, 1:1000), β-Actin (A1978, Sigma, 1:10000) at 4 ℃ overnight. Membranes were washed for 1 hour, incubated with secondary antibodies (anti-rabbit 1:5,000 and anti-mouse 1:5,000) for 1 hr 30 min at room temperature and washed for 6 × 10 min. Each membrane was then placed into detection solution (Bio-RAD, USA), incubated for 2 min at room temperature and detected by ChemiDoc XRS+ (Bio-RAD, USA).

H&E staining

Tissues were weighed, and fixed with 4% paraformaldehyde (Biosesang, Korea) at room temperature (RT) for a week. Paraffin-embedded sections of TA muscle were sliced at thickness of 3 μm. Paraffin sections were deparaffinized, and stained with Hematoxylin & Eosin (H&E), following standard procedures (Autostainer XL, Leica, Germany). Sectioned tissues were analyzed under a scanner (3D HISTECH, Hungary) and Image-Pro program.

Statistical analysis

All values were performed using Prism 7 software. Data were expressed as the mean ± SEM. Statistical analysis was performed using One-way ANOVA between groups. $p < 0.05$ was considered statically significant.

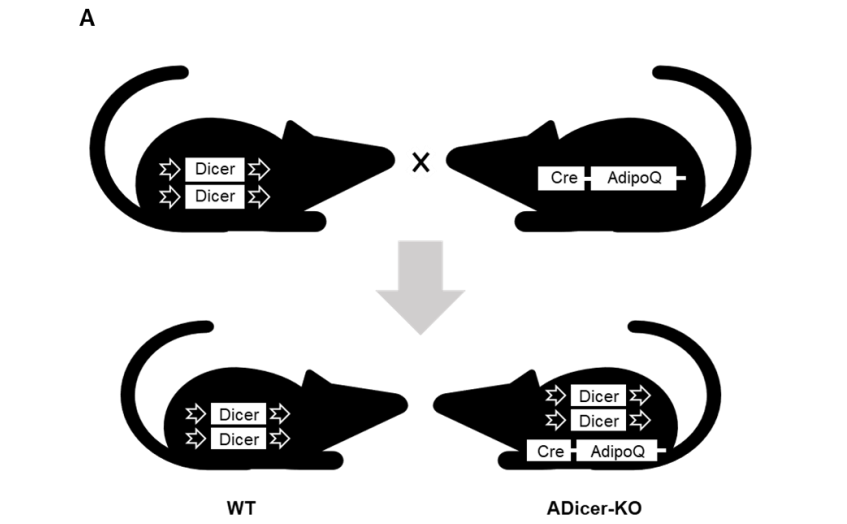
Table 1. Primer sequences for quantitative RT-PCR

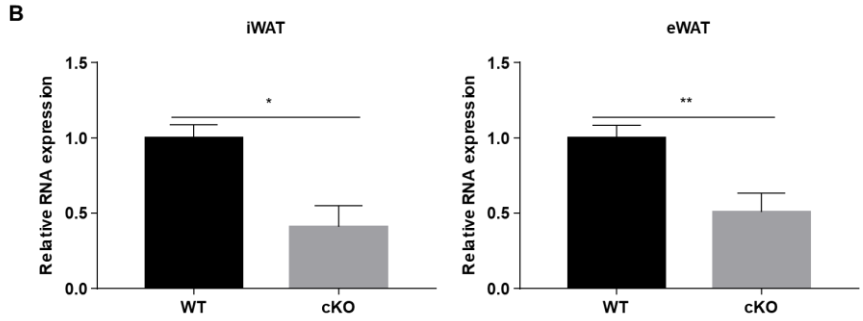
Gene	Sequence
MyoD (forward)	5′ -AGCACTACAGTGGCGACTCA-3′
MyoD (reverse)	5′ -GGCCGCTGTAATCCATCA-3′
Myogenin (forward)	5′ -CAATGCACTGGAGTTCG-3′
Myogenin (reverse)	5′ -ACGATGGACGTAAGGGAGTG-3′
Myf5 (forward)	5′ -CTGCTCTGAGCCCACCAG-3′
Myf5 (reverse)	5′ -GACAGGGCTGTTACATTCAGG-3′
Myh3 (forward)	5′ -CTCTGTCACAGTCAGAGGTGT-3′
Myh3 (reverse)	5′ -TTCCGACTTGCGGAGGAAAG-3′
Myostatin (forward)	5′ -AACCTTCCCAGGACCAGGAGAA-3′
Myostatin (reverse)	5′ -GGCTTCAAAATCGACCGTGAGG-3′
36B4 (forward)	5′ -GAGGAATCAGATGAGGATATGGGA-3′
36B4 (reverse)	5′ -AAGCAGGCTGACTTGGTTGC-3′

3. Results

Dicer expression was decreased in fat from adipose tissue-specific Dicer-knockout (ADicer-KO) mice.

We generated mice specifically lacking Dicer in adipose tissue using Cre-loxP gene recombination system (Fig 1A). Adipose tissue-specific Dicer-knockout mice (ADicer-KO) mice had a defect in miRNA processing in adipose tissue. The mRNA expression of Dicer was decreased in iWAT and eWAT of ADicer-KO mice compared to WT mice (Fig 1B). We confirmed that protein expression was also decreased in BAT, iWAT and eWAT from ADicer-KO mice (Fig 1C).


A
Dicer
Dicer
×
Cre
AdipoQ
Dicer
Dicer
Dicer
Dicer
Cre
AdipoQ
WT
ADicer-KO


B
iWAT
eWAT
Relative RNA expression
1.5
1.0
0.5
0.0
*
**
WT
cKO

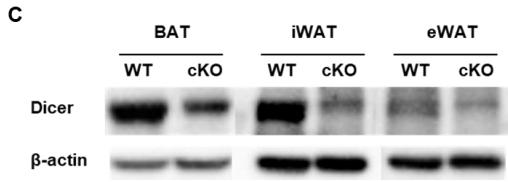

C
BAT
iWAT
eWAT
WT
cKO
WT
cKO
WT
cKO
Dicer
β-actin

Fig 1. Generation of adipose tissue-specific Dicer-knockout (ADicer-KO) mice and Dicer expression in fat.

(A) Schematic showing the creation of ADicer-KO mice. (B) Relative mRNA expression of Dicer measured by qRT-PCR in fat from WT littermates and ADicer-KO mice. (C) The protein expression of in fat from WT littermates and ADicer-KO mice. Values were normalized to 36B4. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

Adipose tissue-derived miRNA didn't have effect on postnatal skeletal muscle generation.

To examine whether adipose tissue-derived miRNA contributed to skeletal muscle growth during early postnatal period in mice, we assessed body composition and cross sectional area of skeletal muscles in postnatal 10 days (P10) mice. There were no significant differences in body weight, fat, and lean mass between WT and ADicer-KO mice (Fig 2A). We analyzed cross sectional area of quadriceps (Quad) and gastrocnemius (Gas) muscles dissected from P10 mice (Fig 2B). There were no significant differences in cross sectional area from two skeletal muscles of both groups (Fig 2C).

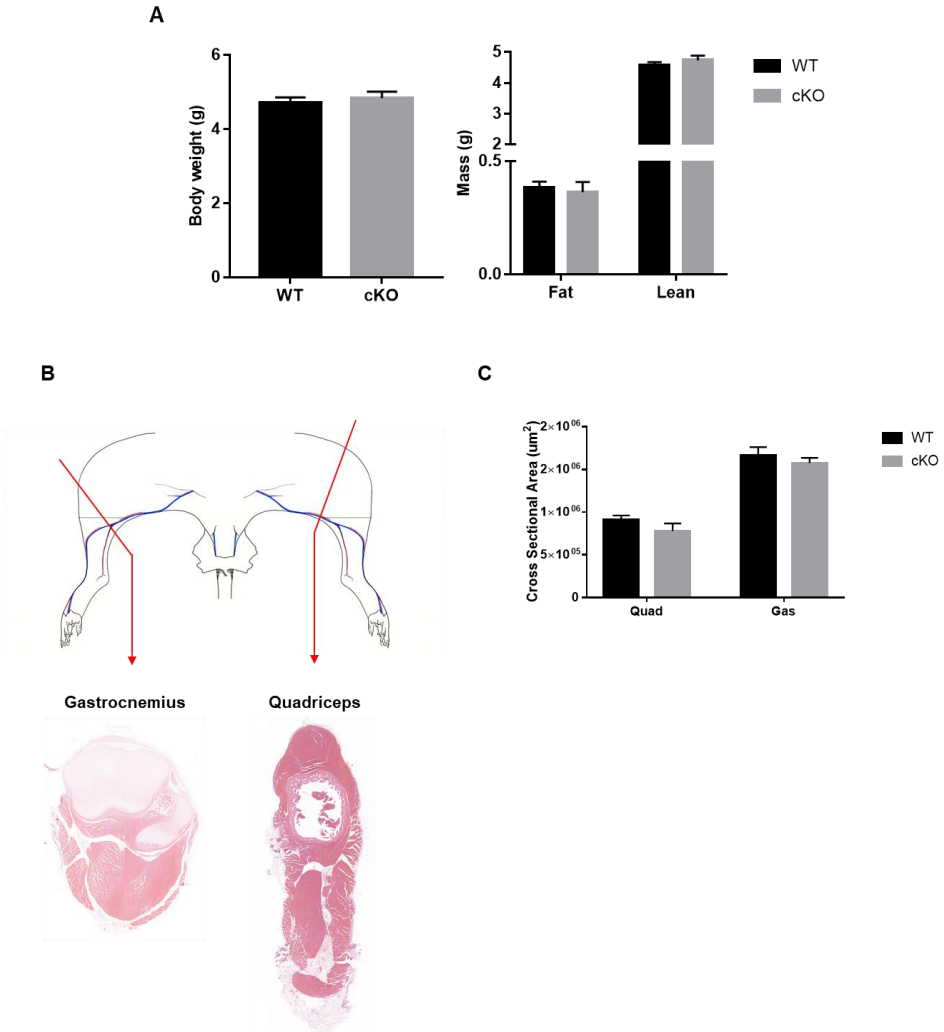
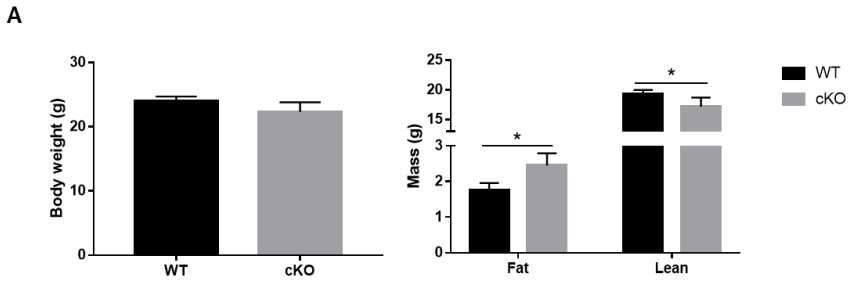


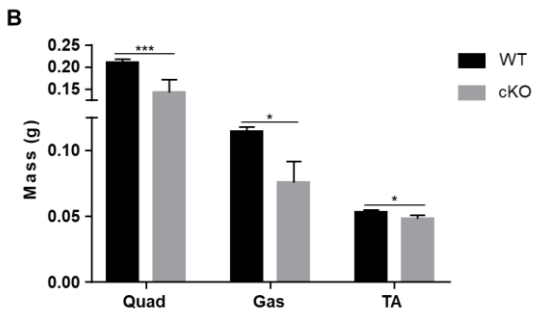
Fig 2. Body composition and cross sectional area of skeletal muscles from postnatal 10 days mice.

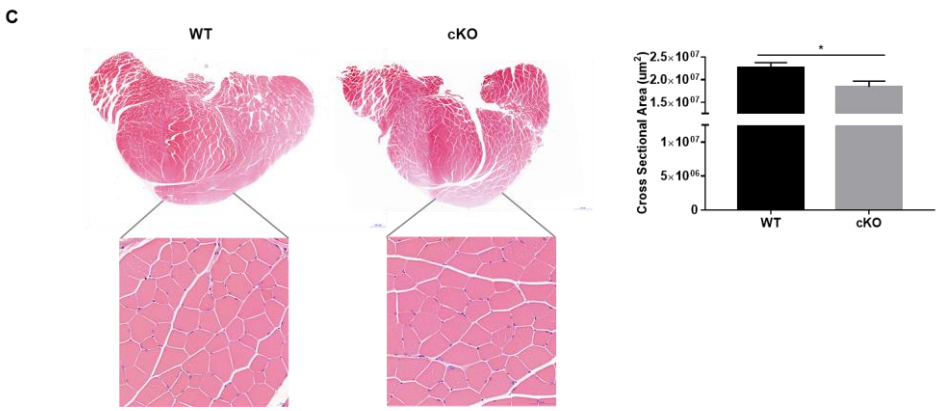
(A) Body weight and body composition in postnatal 10 days (P10) mice. (B) Dissection of quadriceps (Quad) and gastrocnemius (Gas) muscle from P10 mice. (C) Quantitative analysis of cross sectional area from skeletal muscle. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

Deficiency of miRNA from adipose tissue induced defective skeletal muscle growth in adult mice.

We assessed body composition and cross sectional area of 9 weeks old mice to examine whether adipose tissue-derived miRNA has effects on skeletal muscle of adult mice. ADicer-KO mice significantly have more fat mass and less lean mass than WT mice (Fig 3A). We weighed quadriceps (Quad), gastrocnemius (Gas), and tibialis anterior (TA) muscle tissue from both groups. All skeletal muscle weight was decreased in ADicer-KO mice (Fig 3B). We analyzed cross sectional area in the section of quadriceps and gastrocnemius muscles. The cross sectional area from both skeletal muscle tissues was decreased in ADicer-KO mice compared to WT mice (Fig 3C, 3D).


A
Body weight (g)
30
20
10
0
WT
cKO
Mass (g)
25
20
15
3
2
1
0
*
*
Fat
Lean
WT
cKO


B
Mass (g)
0.25
0.20
0.15
0.10
0.05
0.00
***
*
*
Quad
Gas
TA
WT
cKO


C
WT
cKO
Cross Sectional Area (um2)
2.5×10^07
2.0×10^07
1.5×10^07
1×10^07
5×10^06
0
*
WT
cKO

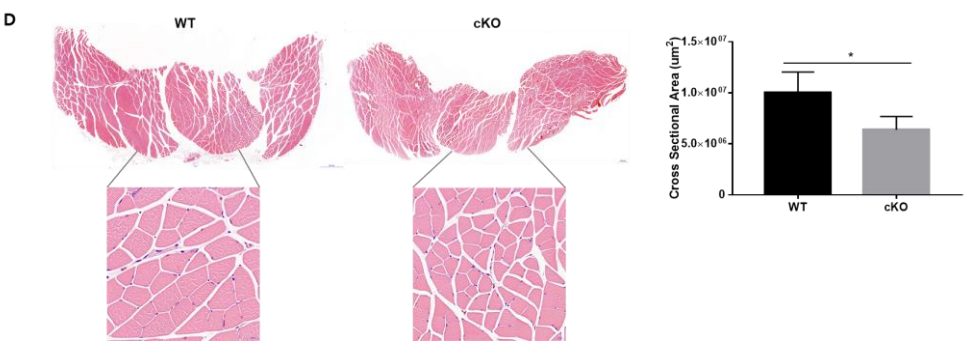

D
WT
cKO
Cross Sectional Area (um2)
1.5×10^07
1.0×10^07
5.0×10^06
0
*
WT
cKO

Fig 3. Body composition and cross sectional area of skeletal muscles from 9 weeks adult mice.

(A) Body weight and body composition in 9 weeks old mice. (B) Quadriceps (Quad), gastrocnemius (Gas) and tibialis anterior (TA) muscle weight. (C) Quantitative analysis of cross sectional area from quadriceps. (D) Quantitative analysis of cross sectional area from gastrocnemius. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

Expression of skeletal muscle synthesis genes was decreased in adult ADicer-KO mice.

We examined the expression of skeletal muscle growth related genes to analyze skeletal muscle homeostasis which means balance between synthesis and degradation. Real-time quantitative PCR was performed to examine the expression of MyoD, Myogenin, Myf5, and Myh3 which regulate proliferation and differentiation. The mRNA expression level of MyoD, Myogenin was reduced in ADicer-KO mice (Fig 4A, 4B). The level of Myf5 and Myh3 expression was downregulated likewise (Fig 4C, 4D). The expression level of Myostatin, which inhibits skeletal muscle cell growth, was also reduced in ADicer-KO mice (Fig 4E). It was thought that the decreased level of Myostatin was because there was no need to suppress skeletal muscle growth since the expression of genes synthesizing skeletal muscles was initially low. ADicer-KO mice showed low expression levels in both the myogenesis genes and the gene that suppresses it, which means that they have impaired muscle homeostasis.

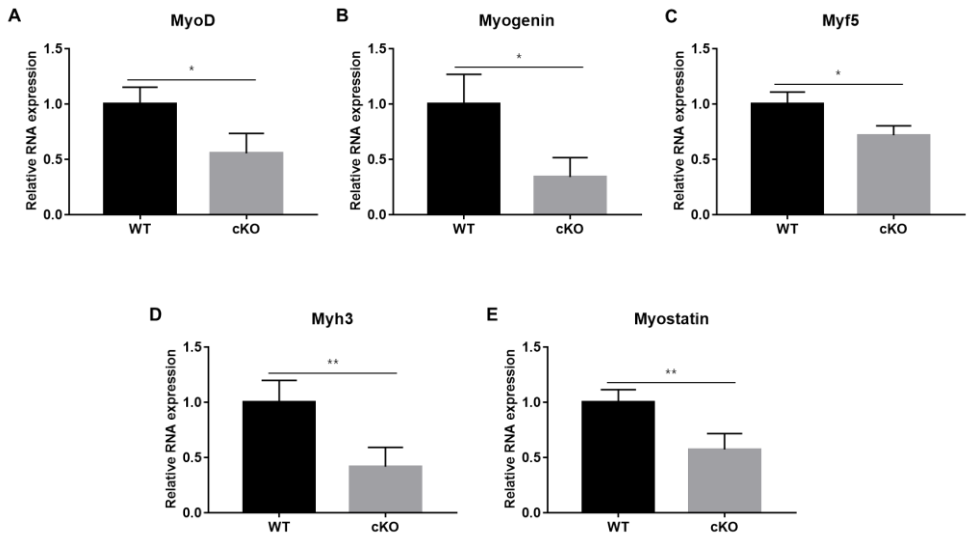


Fig 4. Decreased expression of skeletal muscle synthesis genes in adult ADicer-KO mice.

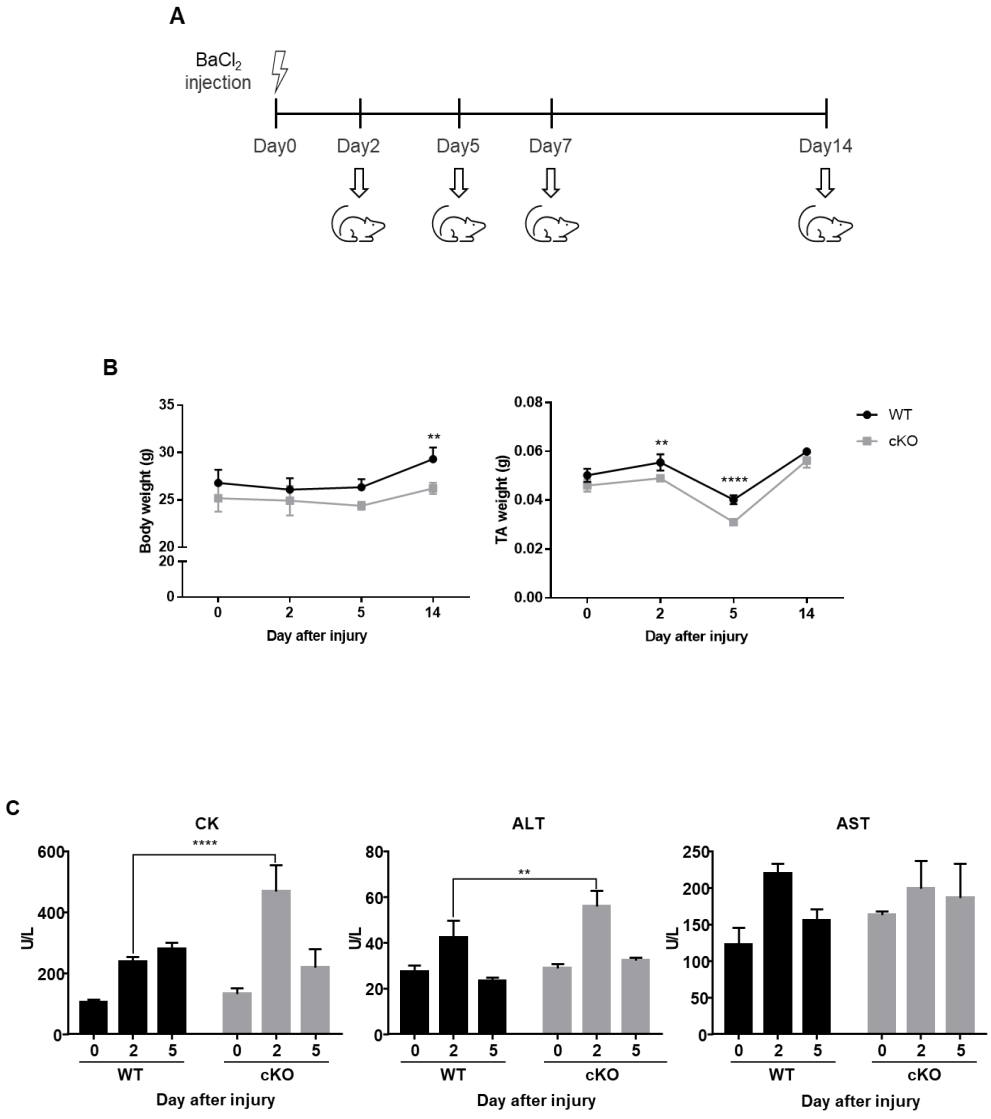
(A) Relative mRNA expression levels of MyoD in TA measured by qRT-PCR in ADicer-KO mice and WT littermates. (B) Relative mRNA expression levels of Myogenin in TA measured by qRT-PCR. (C) Relative mRNA expression levels of Myf5 in TA measured by qRT-PCR. (D) Relative mRNA expression levels of Myh3 in TA measured by qRT-PCR. (E) Relative mRNA expression levels of Myostatin in TA measured by qRT-PCR. Values were normalized to 36B4. ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$)

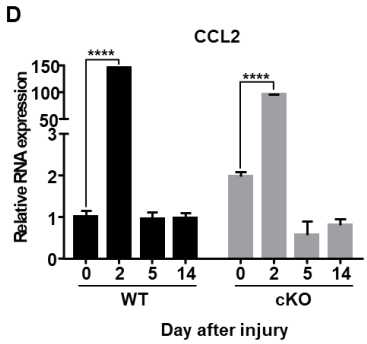
Deficiency of miRNA from adipose tissue induced delayed skeletal muscle regeneration.

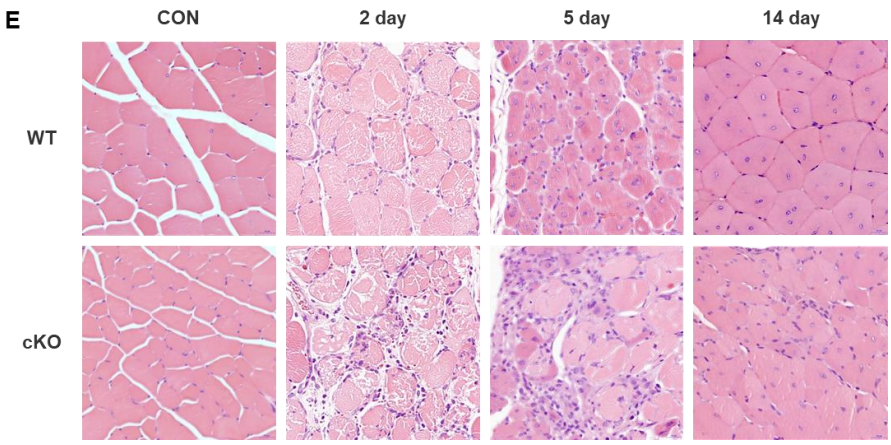
To examine the regeneration process in TA muscle, we harvested TA muscle at 2, 5, 7, and 14 days after $BaCl_2$-induced injury (50 μL) (Fig 5A). We weighed body and tissue weight by date (Fig 5B). Body weight and TA weight from ADicer-KO mice was lower than WT mice (Fig 5B). There was a TA weight loss at 5 days in both groups and it was recovered at 14 days as the regeneration progressed. Especially, there was a significant weight loss in ADicer-KO TA muscle at 5 days. We analyzed biochemical markers from serum at 2 and 7 days after $BaCl_2$ injection, with uninjured mice (control, CON) (Fig 5C). Significant increase in serum CK level indicative of myoglobinemia was observed in both groups, compared to level in the uninjected controls. It showed that CK level was increased higher in ADicer-KO mice at 2 days after injury. There was similar pattern of increased ALT and AST levels, tracking with serum CK levels, suggesting that the increase in ALT and AST levels was due to acute myonecrosis. Also, CCL2 expression was highly increased at 2 days in both groups (Fig 5D).

We performed hematoxylin and eosin staining to see the skeletal muscle fibers during the regeneration (Fig 5E). Fiber bundle was

disrupted and mononuclear infiltration peaked at 2 days after injury in both groups. Myofibers were observed with numerous mononuclear cells, largely inflammatory and skeletal muscle precursor cells. It showed that average cross sectional area of a skeletal muscle fiber at 14 days after injury was dramatically reduced in regenerating skeletal muscles of ADicer-KO mice (Fig 5F). Also, regenerated fibers which had nuclei in a central position appeared by 5 days after injection. Cross sectional area of these regenerating fibers including two or more centralized nuclei were significantly reduced in ADicer-KO mice compared with WT mice at 5 and 14 days after injection (Fig 5G). The size of skeletal muscle fibers from ADicer-KO mice was smaller than those of WT after injury. They showed delayed skeletal muscle regeneration process because of impaired regeneration ability.


A
BaCl2 injection
Day0
Day2
Day5
Day7
Day14
B
Body weight (g)
Day after injury
TA weight (g)
Day after injury
WT
cKO
C
CK
ALT
AST
U/L
WT
cKO
Day after injury


D
CCL2
Relative RNA expression
150
100
50
3
2
1
0
****
****
0 2 5 14 0 2 5 14
WT
cKO
Day after injury


E
CON
2 day
5 day
14 day
WT
cKO

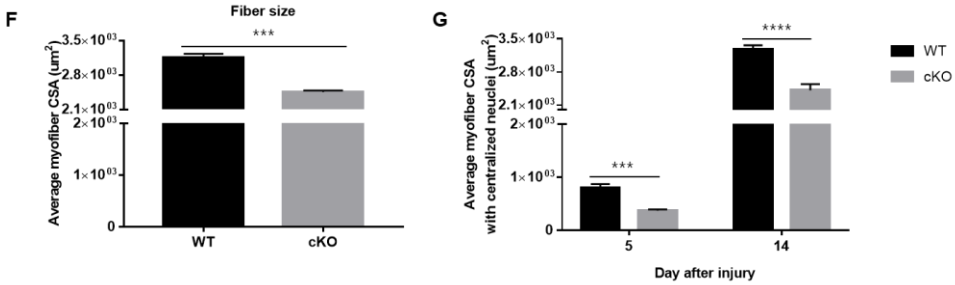

F
Fiber size
***
Average myofiber CSA (um2)
3.5×10^03
2.8×10^03
2.1×10^03
2×10^03
1×10^03
0
WT
cKO
G
****
***
Average myofiber CSA with centralized neuclei (um2)
5
14
Day after injury
WT
cKO

Fig 5. Impaired skeletal muscle regeneration in the absence of adipose tissue-derived miRNA.

(A) Barium chloride ($BaCl_2$) induced skeletal muscle injury experimental procedure in TA. (B) Body weight and TA weight. (C) Analysis of serum biochemical markers from mice at 2 and 5 days after injury, with uninjured mice (control). CK, creatinine kinase; ALT, alanine aminotransferase; AST, aspartate aminotransferase. (D) Relative mRNA expression levels of CCL2 measured by qRT-PCR. (E) H&E-stained sections of TA muscle at 2, 5, and 14 days after $BaCl_2$ injection, with uninjured skeletal muscle (control, CON). (F) Average size of myofibers at 14 days after injury. (G) Average size of regenerating myofibers with centralized nuclei at 5 and 14 days after injury. Values were normalized to 36B4. ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$)

Expression of myogenic regulatory factors (MRFs) was decreased in ADicer-KO mice during regeneration.

To determine the myogenic regulatory factors (MRFs) expression during the regeneration period. Real-time quantitative PCR was performed. Results showed that MyoD and Myogenin was increased during the time in WT and ADicer-KO mice (Fig 6A, 6B). At 2 days, ADicer-KO mice showed the higher expression of MyoD than WT mice. However, WT mice showed the much higher expression levels than ADicer-KO mice at 14 days. Myf5 was inactivated at 2 days and then activated in both groups thereafter (Fig 6C). MRFs expression was significantly increased in 14 days when compared to uninjured skeletal muscles and ADicer-KO mice showed lower expression of MRFs than WT mice followed by delayed regeneration process during the time.

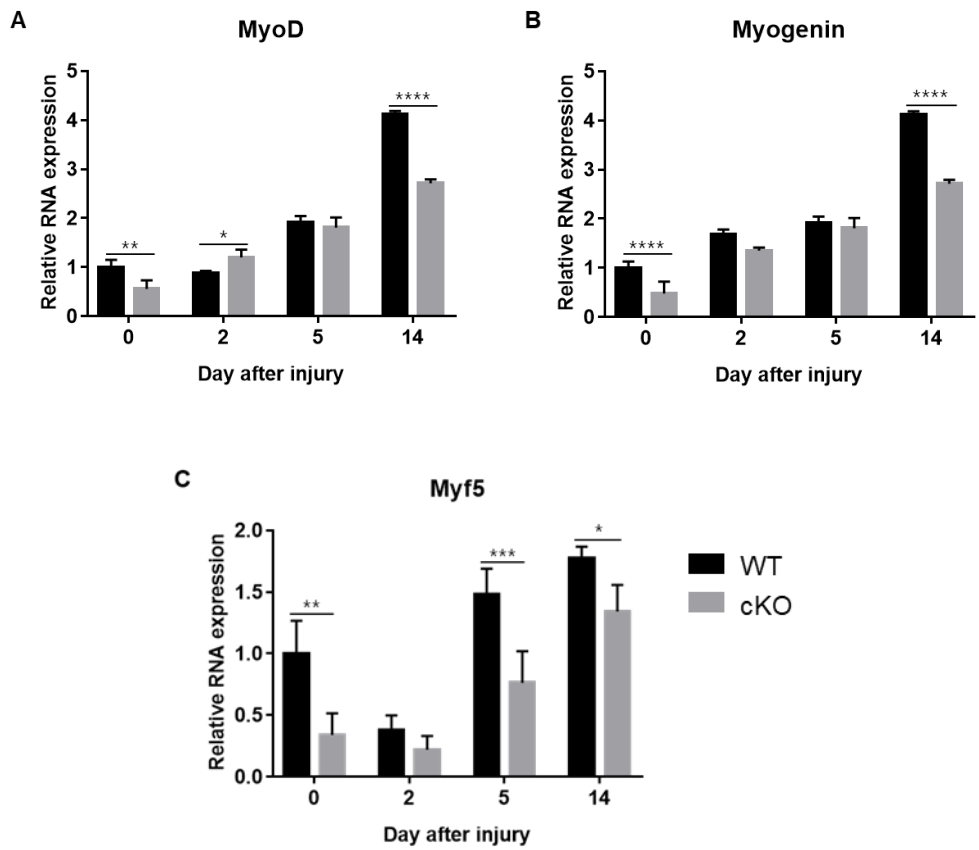


Fig 6. Decreased expression level of MRFs in ADicer-KO mice during regeneration.

(A) Relative mRNA expression levels of MyoD measured in TA by qRT-PCR in ADicer-KO mice and WT littermates. (B) Relative mRNA expression levels of Myogenin measured by qRT-PCR. (C) Relative mRNA expression levels of Myf5 measured by qRT-PCR. Values were normalized to 36B4. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

4. Discussion

Several recent studies have proposed that fat-specific miRNA circulates and have effects on other distant organs such as liver and skeletal muscle [22, 14, 23]. Thus, we generated ADicer-KO mice and confirmed that the mRNA and protein expression of Dicer was decreased in fat tissues compared to WT mice. We examined the body composition and skeletal muscle cross sectional area in postnatal 10 days and 9 weeks old adult mice. There are significant differences between ADicer-KO and WT not in postnatal 10 days mice, but in 9 weeks old adult mice. Skeletal muscle weight and cross sectional area were decreased in ADicer-KO mice compared to WT mice. We confirmed that the expression of genes related to skeletal muscle synthesis was reduced. Myostatin, a gene that inhibits skeletal muscle growth, was also decreased. It was thought that the decreased level of myostatin was because there was no need to suppress skeletal muscle growth since the expression of genes trying to grow skeletal muscles was initially low.

Skeletal muscles allow voluntary movement and they play an important role in regulating whole body metabolism and homeostasis [24, 25]. Also, they have a remarkable ability for

regeneration in response to injury. Skeletal muscle-derived miRNAs have been shown to play an important role in human embryonic myogenesis and also in adult myogenesis after injury. miR-133 and -27 involve in proliferation, and miR-206 and -1 involve in myoblast differentiation [22, 23]. We showed that skeletal muscle regeneration after BaCl2-induced injury was delayed in mice with the loss of Dicer in adipose tissue. The cross sectional area of regenerating myofiber in TA muscle was dramatically reduced in ADicer-KO mice compared to WT mice. This defective regeneration was mainly ascribed to the downregulation of myogenic regulatory factors (MRFs) during the repair process. It's not well known about the effects of adipose tissue on development and regeneration of skeletal muscle. More findings are needed to identify which specific miRNA derived from adipose tissue targets the MRFs in the skeletal muscle.

miRNAs play a major role in maintaining cell identity and have been shown to play important roles in both white and brown fat differentiation [11, 26]. The expression of miRNAs was altered in response to obesity, insulin resistance, or cold exposure [10, 27]. Previous study showed that adipose tissue-specific Dicer-knockout (ADicer-KO) mice have a phenotype of lipodystrophy characterized by decreased WAT mass and increased whitening

BAT mass [13]. ADicer−KO mice had significant changes in 422 exosomal miRNAs. Of these, 3 miRNAs were significantly increased, while 419 miRNAs had significant decreases with 88% reduced by >4−fold. In ADicer−KO mice, there was a 57%-74% decrease in circulating adiponectin and a nearly 50% reduction in serum leptin [13]. As deficiency of miRNA in adipose tissue influenced the level of adipokine, we couldn' t exclude the effect of adipokine on skeletal muscle regeneration after injury.

The capacity for regeneration depends not only on MRFs but also on muscle stem cells (MSCs), reside between the sarcolemma and the basal lamina in a quiescent state [28]. In response to skeletal muscle injury, Pax7+ satellite cells (SCs) are activated to proliferate, differentiate, and repair damaged region. Some of them undergo self−renewal process, which means that activated SCs reverse to quiescence to replenish the stem cell pool [29, 30]. Abnormal SC pool maintenance reduces capability of regeneration and impairs the homeostasis of skeletal muscle [31, 32]. It should be determined whether the depletion of fat−specific miRNA cause dysfunction of skeletal muscle satellite cells during regeneration that results in delayed repair process.

In summary, we revealed that deficiency of miRNA from adipose tissue caused impaired skeletal muscle development and

delayed regeneration process resulted from downregulation of MRFs. These findings raised the possibility that adipose tissue-derived miRNAs can act as regulators of myogenesis in skeletal muscle providing a new mechanism of cell-cell crosstalk. It is well known about the effects of skeletal muscle-derived miRNAs on skeletal muscle itself. However, there was no research proposing that miRNA-derived from adipose tissue affects skeletal muscle. Our study first revealed that adipose tissue-derived miRNA affects skeletal muscle by regulating MRFs in skeletal muscle.

5. References

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국문초록

지방 조직 유래 miRNA가 근육의 발달 및 재생에 미치는 영향

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지방 조직은 에너지 저장을 위한 주요 기관이며 신진 대사, 면역, 그리고 항상성 유지 등을 담당한다. 지방 조직에서는 exosome에 둘러싸여 수많은 miRNA가 방출되는데, 이들은 혈액을 통해 순환하며 멀리 떨어져 있는 다른 장기를 연결함으로써 전신 대사 조절에 중요한 역할을 한다. 지방 조직에서 방출된 miRNA가 지방 조직 자체에 미치는 영향에 대해서는 잘 알려져 있지만, 골격근에 미치는 영향에 대해서는 현재까지 보고된 바가 없다. 우리는 지방 조직 특이적 Dicer 결핍 (Adipose tissue-specific Dicer-knockout) 마우스 모델을 이용하여 지방 조직 유래 miRNA가 골격근에 미치는 영향에 관해 연구하였다. 신체 구성 측정과 근육 단면적 측정을 통해 9주령의 지방 조직 특이적 Dicer 결핍 마우스는 정상 마우스에 비해 더 작은 골격근을 가지는

것을 확인하였고, TA 근육에서 근생성과 관련된 유전자의 발현이 감소되어 있었다. $BaCl_2$를 통해 TA 근육 부상을 유도한 후 재생 과정을 확인하였을 때, 지방 조직 특이적 Dicer 결핍 마우스는 정상 마우스에 비해 근원성 조절 인자들 (Myogenic regulatory factors)의 발현이 감소되었다. 또한, 지방 조직 특이적 Dicer 결핍 마우스는 더 작은 근섬유를 가지며 근육 재생 속도가 지연되었다는 것을 확인하였다. 이러한 결과들을 통해 본 연구는 골격근 발달에 지방 조직 유래 miRNA가 미치는 영향을 보여주며 지방 조직과 골격근 사이의 잠재적인 연관성을 제공한다.