BMB
Reports

BMB Rep. 2020; 53(11): 605-610
www.bmbreports.org

MiR-183-5p induced by saturated fatty acids regulates the myogenic differentiation by directly targeting FHL1 in C2C12 myoblasts

Mai Thi Nguyen[1], *Kyung-Ho Min*[1] *& Wan Lee*[1,2,*]

[1]Department of Biochemistry, Dongguk University College of Medicine, Gyeongju 38066, [2]Channelopathy Research Center (CRC), Dongguk University College of Medicine, Goyang 10326, Korea

Skeletal myogenesis is a complex process that is finely regulated by myogenic transcription factors. Recent studies have shown that saturated fatty acids (SFA) can suppress the activation of myogenic transcription factors and impair the myogenic differentiation of progenitor cells. Despite the increasing evidence of the roles of miRNAs in myogenesis, the targets and myogenic regulatory mechanisms of miRNAs are largely unknown, particularly when myogenesis is dysregulated by SFA deposition. This study examined the implications of SFA-induced miR-183-5p on the myogenic differentiation in C2C12 myoblasts. Long-chain SFA palmitic acid (PA) drastically reduced myogenic transcription factors, such as myoblast determination protein (MyoD), myogenin (MyoG), and myocyte enhancer factor 2C (MEF2C), and inhibited FHL1 expression and myogenic differentiation of C2C12 myoblasts, accompanied by the induction of miR-183-5p. The knockdown of FHL1 by siRNA inhibited myogenic differentiation of myoblasts. Interestingly, miR-183-5p inversely regulated the expression of FHL1, a crucial regulator of skeletal myogenesis, by targeting the 3′UTR of FHL1 mRNA. Furthermore, the transfection of miR-183-5p mimic suppressed the expression of MyoD, MyoG, MEF2C, and MyHC, and impaired the differentiation and myotube formation of myoblasts. Overall, this study highlights the role of miR-183-5p in myogenic differentiation through FHL1 repression and suggests a novel miRNA-mediated mechanism for myogenesis in a background of obesity. [BMB Reports 2020; 53(11): 605-610]

*Corresponding author. Tel: +82-54-770-2409; Fax: +82-54-770-2447; E-mail: wanlee@dongguk.ac.kr

https://doi.org/10.5483/BMBRep.2020.53.11.175

Received 24 August 2020, Revised 11 October 2020, Accepted 22 October 2020

Keywords: Differentiation, FHL1, MicroRNA, miR-183-5p, Palmitic acid

INTRODUCTION

The maintenance of muscle mass and integrity by myogenesis is essential for whole-body homeostasis as well as for the proper physical and metabolic functions of muscle (1). Skeletal myogenesis is a highly coordinated complex process involving satellite cell activation, myoblast proliferation, cell-cycle exit, and the fusion of myoblasts into multinucleated myotubes (1). During this process, myogenic transcription factors, such as myoblast determination protein (MyoD) and myogenin (MyoG), play a vital role in the differentiation of progenitor cells into myotubes (2). In addition, the myocyte-specific enhancer factor 2 (MEF2) family of transcription factors, which promote the myogenic differentiation of progenitor cells into myotubes, are also important regulators of skeletal muscle differentiation (2). On the other hand, dysregulation of myogenesis leads to diverse pathological conditions, including metabolic myopathy, muscular dystrophy, and muscle wasting (3). The excessive intake of saturated fatty acids (SFA) has been reported to increase intramuscular fat deposition and induce lipotoxicity, such as mitochondrial dysfunction, apoptosis, and inflammation, which eventually leads to muscle atrophy and wasting (4). Recent studies have also shown that SFA can impair the activation of myogenic factors and suppress myogenic differentiation in various progenitor cells (5-8). However, the molecules involved and the mechanisms responsible for their inhibition of myogenic differentiation in SFA-treated myoblasts remains substantially unknown.

MicroRNAs (miRNAs) are a large family of highly conserved small non-coding RNAs that function as negative regulators in gene expression by binding to the 3′UTRs of target mRNAs (9). Although the targets and roles of most miRNAs are largely unknown, accumulating evidence suggests that they are widely involved in normal and pathophysiological cellular processes (9). Therefore, dysregulation of miRNAs expression is closely linked to many diseases, including diabetes, neurodegeneration, and cancer (10). Over the last few decades, a growing body of research has shown that miRNAs play an essential role in myogenesis and have a significant impact on muscle homeostasis, muscle mass maintenance, and pathogenesis of myo-

ISSN: 1976-670X (electronic edition)

pathies (11). Moreover, many studies have shown that many miRNAs are dysregulated in obesity and suggested that exosomal miRNAs could be a new class of endocrine factors (12, 13). Despite the advances in knowledge on myogenesis, the underlying mechanism for how obesity-induced miRNAs are linked to the impaired myogenic differentiation is poorly understood.

The four and a half LIM domains protein 1 (FHL1) encoded by the FHL1 gene is a member of the FHL protein family and is involved in nuclear-cytoplasmic communication, muscle development sarcomere assembly (14, 15). Since FHL1 is highly expressed in skeletal muscle, an increasing number of studies have focused on the role of FHL1 in skeletal muscle integrity and myopathies. Mutations in the FHL1 gene have been reported to be associated with various human myopathies, including X-linked myopathy with postural muscle atrophy (16), scapuloperoneal myopathy (17), and Emery–Dreifuss muscular dystrophy (18). Moreover, recent studies have shown that FHL1 is a crucial regulator of skeletal myogenesis and muscle maintenance (19-22). However, although FHL1 plays an essential role in myogenesis, the association between FHL1 expression and SFA accumulation has not been explored. Furthermore, it is unclear if specific miRNAs induced by SFA suppresses FHL1 expression, thereby leading to an impairment of myogenic differentiation.

In this study, we examined whether SFA inhibits myogenic differentiation and FHL1 expression in C2C12 myoblasts. We also explored the functional role and regulation of FHL1 and miR-183-5p on myogenic differentiation and myotube formation in myoblasts. This study shows the implications of miR-183-5p on myogenesis and suggests a novel mechanism for miRNA-mediated myogenic regulation.

RESULTS

PA inhibits myogenic differentiation and augments miR-183-5p expression

To determine the effects of PA, the most abundant dietary SFA, on myogenic differentiation, C2C12 myoblasts were pretreated with PA (0.1 mM, 24 hr) before differentiation. Then, myogenic differentiation and the expression of myogenic factors, such as MyoD, MyoG, MEF2C, and MyHC, were analyzed until day 5. Based on the differentiation index, fusion index, and MyHC-positive areas in immunocytochemistry (Fig. 1A-D), PA reduced the differentiation and myotube formation of C2C12 myoblasts drastically, indicating that PA impaired the myogenic differentiation of myoblasts. Subsequently, the expression of myogenic factors was determined in the control and PA-treated myoblasts by immunoblot analysis. As shown in Fig. 1E, the levels of MyoD, MyoG, MEF2C, and MyHC expression were reduced significantly by PA. Therefore, the impairment of myogenic differentiation in the PA-treated myoblasts was primarily due to the suppression of myogenic factors.

Since FHL1 was reported to play a critical role in skeletal

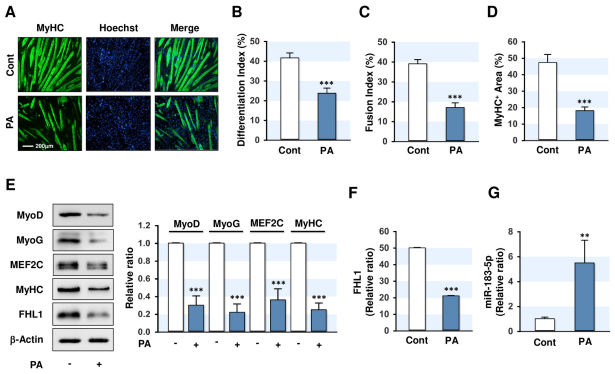

Fig. 1. PA impairs myogenic differentiation and upregulates miR-183-5p in C2C12 myoblasts. To examine the effect of PA on myoblast differentiation, C2C12 myoblasts were treated with PA (0.1 mM) for 24 hr and differentiated for five days. (A) Immunofluorescence staining with a specific antibody against MyHC (green). Hoechst stains nuclei. Scale bar: 200 μm. (n = 6) (B-D) Quantified differentiation index, fusion index, and MyHC-positive area according to Methods (n = 6). (E) Representative immunoblots of myogenic factors after differentiation for three days (n = 3). (F) Expression of FHL1 (n = 3). The level of expression was normalized to the amount of β-Actin. (G) *q*RT-PCR of miR-183-5p after 24 hr with PA (n = 4). The level of expression was normalized to the amount of U6. Values are means ± S.E.M. $^{**}P < 0.01$; $^{***}P < 0.001$ vs Cont.

myogenesis (19-22), this study examined whether PA suppresses the expression of FHL1 in C2C12 cells. Interestingly, FHL1 expression in myoblasts was also reduced significantly by PA (Fig. 1E, F), implying that the reduction of FHL1 expression is associated with the suppression of myogenic factors and myogenic differentiation by PA. To determine how PA suppressed FHL1 expression, we investigated whether miRNAs induced by PA might control FHL1 expression and myogenic differentiation in myoblasts. Based on the miRNA microarray analysis in PA-treated myoblasts, many miRNAs were upregulated by PA (data not shown). Among those, miR-183-5p was selected for further experiments because *in silico* analysis predicted FHL1 as a target of miR-183-5p. Using *q*RT-PCR analysis for verifying the microarray results, the level of miR-183-5p expression was found to be increased significantly in PA-treated C2C12 myoblasts (Fig. 1G). Overall, PA downregulated the expression of myogenic factors and inhibited the myogenic differentiation of C2C12 myoblasts, accompanying FHL1 reduction and miR-183-5p induction.

miR-183-5p directly targets the 3′UTR of FHL1

Because miR-183-5p expression was found to be inversely related to the FHL1 level in PA-treated myoblasts, this study next examined whether miR-183-5p directly targets FHL1. *In silico* target prediction analysis, such as TargetScan and miRWalk, predicted FHL1 to be a possible target of miR-183-5p, because the 3′UTR region of FHL1 contains a potential binding site for miR-183-5p (Fig. 2A). To confirm direct targeting of

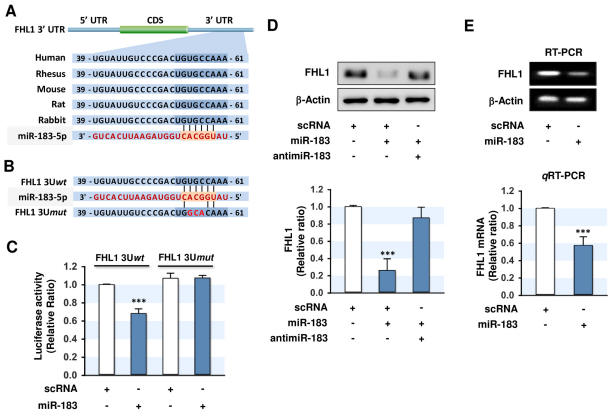

Fig. 2. MiR-183-5p directly targets to the FHL1 3′UTR and reduces FHL1 expression. (A) Sequence alignment of the 3′UTR fragments of FHL1 with miR-183-5p. (B) Sequences of the wild-type (FHL1 3U*wt*) and mutant (FHL1 3U*mut*) of FHL1 3′UTR. (C) Luciferase reporter assays using the 100 nM of scRNA or miR-183-5p with the 100 nM of a segment of wild-type (FHL1 3U*wt*) or mutant (FHL1 3U*mut*) FHL1 3′UTR in pmirGLO vectors (n = 4). (D) Immunoblot analysis (n = 3) of FHL1 at 24 hr after transfection with the 100 nM of scRNA control, miR-183-5p mimic (miR-183), or antimiR-183-5p (antimiR-183). (E) RT-PCR (upper) and *q*RT-PCR (lower) analysis of FHL1 expression at 24 hr after transfection with the 100 nM of scRNA control or miR-183-5p mimic (miR-183) (n = 3). The level of expression was normalized to the amount of β-Actin. Values are means ± S.E.M. ***P < 0.001 vs scRNA.

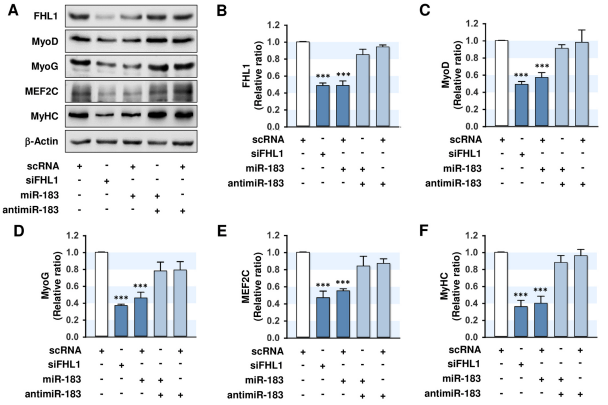

Fig. 3. MiR-183-5p suppresses the expression of myogenic factors as well as FHL1. C2C12 myoblasts were transfected with the 100 nM of scRNA control, siRNA of FHL1 (siFHL1), miR-183-5p mimic (miR-183), or antimiR-183-5p (antimiR-183). (A) Representative immunoblots of myogenic factors after differentiation for three days (n = 3). (B) Quantitative analysis of FHL1 expression (n = 3). (C-F) Quantitative analysis of myogenic genes, including MyoD, MyoG, MEF2C, and MyHC (n = 3). The level of expression was normalized to the amount of β-Actin. Values are means ± S.E.M. ***P < 0.001 vs scRNA.

miR-183-5p on the FHL1 3′UTR, we subcloned the wild-type (FHL1 3U*wt*) and mutant (FHL1 3U*mut*) 3′UTR segments of FHL1 into the pmirGLO vector (Fig. 2B), and then C2C12 myoblasts were cotransfected with the pmirGLO vector and either the mature miR-183-5p mimic or scRNA control. In cells cotransfected with miR-183-5p mimic and the pmirGLO vector containing the wild-type FHL1 3′UTR attenuated the luciferase activity significantly compared to the scRNA (Fig. 2C). However, the luciferase activity was unaffected in the cells cotransfected with miR-183-5p mimic and the FHL1 3U*mut* pmirGLO vector (Fig. 2C). Thus, miR-183-5p directly binds to the FHL1 3′UTR. To confirm this further, the protein and mRNA expression of FHL1 was determined in C2C12 myoblasts transfected with miR-183-5p mimic or scRNA. The transfection of the miR-183-5p mimic decreased the protein level of FHL1 in C2C12 myoblasts significantly, as compared to control (Fig. 2D). In addition, cotransfection with antimiR-183-5p, an inhibitor of miR-183-5p, almost entirely abolished the inhibitory effect of miR-183-5p mimic on FHL1 protein expression (Fig. 2D). Moreover, the level of FHL1 mRNA was also decreased by the transfection of miR-183-5p mimic (Fig. 2E). These results suggest that miR-183-5p negatively regulates the expression of FHL1 through direct targeting to FHL1 3′UTR.

Overexpression of miR-183-5p inhibits the myogenic differentiation of C2C12 myoblasts

As miR-183-5p regulates the expression of FHL1, this study examined whether FHL1 reduction or miR-183-5p upregulation impairs the expressions of myogenic factors in myoblasts (Fig. 3). C2C12 myoblasts were transfected with the 100 nM of scRNA, FHL1 siRNA, miR-183-5p mimic, or antimiR-183-5p, and harvested in a differentiation medium for five days. siRNA against FHL1 was used to knockdown the expression of FHL1. Transfection of FHL1 siRNA suppressed the protein expression of FHL1 by approximately 60% (Fig. 3A, 3B). As expected, the knockdown of FHL1 highly decreased myogenic factors, such as MyoD, MyoG, MEF2C, and MyHC, in C2C12 myoblasts (Fig. 3C-F). Interestingly, transfection of miR-183-5p mimic also reduced the expression of myogenic factors in C2C12 myoblasts significantly with the concomitant suppression of FHL1 compared to the scRNA-transfected control (Fig. 3). Cotransfection with antimiR-183-5p has almost restored the inhibitory effects of miR-183-5p on the expression of myogenic factors (Fig. 3). Thus, induction of miR-183-5p caused the downregulation of FHL1 and also suppressed the expressions of MyoD, MyoG, MEF2C, and MyHC in C2C12 myoblasts.

Next, we determined whether the upregulation of miR-183-5p inhibits myogenic differentiation and myotube formation. Differentiation was assessed by immunocytochemistry with the MyHC antibody and Hoechst and subsequently subjected to quantitative analysis. As expected, the knockdown of FHL1 using siRNA drastically inhibited the myotube formation of C2C12 cells (Fig. 4A). Quantitative analysis, such as the differentiation

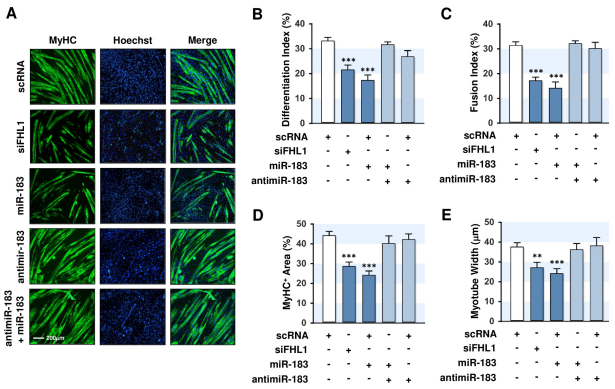

Fig. 4. MiR-183-5p inhibits myogenic differentiation. C2C12 myoblasts were transfected with the 100 nM of scRNA control, siRNA of FHL1 (siFHL1), miR-183-5p mimic (miR-183), or antimiR-183-5p (antimiR-183) and differentiated for five days. (A) Immunofluorescence staining with a specific antibody against MyHC (green). Hoechst stains nuclei. Scale bar: 200 μm. (n = 5) Quantitative analysis of differentiation index (B), fusion index (C), MyHC positive area (D), and myotube width (E) according to Methods. Values are means ± S.E.M (n = 5). **P < 0.01; ***P < 0.001 vs scRNA.

index, fusion index, MyHC-positive area, and myotube width, clearly showed that the knockdown of FHL1 expression with siRNA caused the impaired myogenic differentiation of C2C12 myoblasts (Fig. 4B-E). Similarly, transfection of the miR-183-5p mimic instead of FHL1 siRNA impaired the differentiation of C2C12 myoblasts based on immunocytochemistry (Fig. 4A) and quantitative analysis of myogenic differentiation (Fig. 4B-E). Moreover, cotransfection with antimiR-183-5p almost entirely blocked the miR-183-5p mimic-induced inhibitions of differentiation and myotube formation (Fig. 4A-E). Therefore, these results suggest that the induction of miR-183-5p impairs the myogenic differentiation and myotube formation in C2C12 myoblasts.

DISCUSSION

Despite the growing interest in the roles of miRNAs in myogenesis and muscle homeostasis, the target molecules and myogenic regulatory mechanisms of miRNAs are mostly unknown. This study examined the effects of SFA-induced miR-183-5p on the myogenic differentiation in C2C12 myoblasts. The key contributions of this study to the current knowledge are as follows: (i) PA suppresses FHL1 expression and myogenic differentiation in C2C12 myoblasts. (ii) PA upregulates the miR-183-5p expression in myoblasts. (iii) The knockdown of FHL1 inhibits the expressions of myogenic factors and differentiation. (iv) miR-183-5p targets 3′UTR of FHL1 directly, thereby downregulating FHL1. (v) The transfection of miR-183-5p mimic suppresses the expression of myogenic genes and impairs the differentiation and fusion of myoblasts. Therefore, these results highlight the potential role of miR-183-5p in myogenesis through the repression of FHL1 and suggest a novel miRNA-mediated mechanism in the myogenic regulation by SFA and obesity.

The accumulation of SFA in skeletal muscle is known to induce lipotoxicity, such as mitochondrial dysfunction and apoptosis, which is closely associated with muscle atrophy and wasting (4, 23). Recently, it has been recognized that SFA could inhibit the activation of myogenic transcription factors and impair myogenic differentiation in various progenitor cells (4, 23). Consistent with previous findings, we have shown that PA suppressed the myogenic differentiation of C2C12 myoblasts accompanied by a significant reduction of myogenic transcription factors. Furthermore, this study revealed that PA suppressed the expression of FHL1 and upregulated the expression of miR-183-5p in C2C12 myoblasts (Fig. 1).

Although defects in the FHL1 gene are linked to multiple human myopathies (14, 15), the roles and modulation of FHL1 in skeletal muscle are poorly understood. However, several lines of evidence suggest that FHL1 plays an essential role in skeletal muscle myogenesis and homeostasis. Transgenic mice carrying the FHL1 gene exhibited skeletal muscle hypertrophy and a fiber-type switch, resulting in increased whole-body strength and fatigue resistance (19). Similarly, the overexpression of FHL1 increased myotube fusion and caused myotube hypertrophy in C2C12 cells, suggesting that FHL1 enhances skeletal myogenesis and muscle mass (19, 20). On the other hand, Han *et al*. recently reported that the knockdown of FHL1 decreased the myogenic markers, such as MyoD, MyoG, and MyHC, and reduced myotube formation in chicken primary myoblasts (24). In addition, the silencing of FHL1 increased Atrogin-1 and MuRF1, which led to muscle atrophy and wasting, unveiling the crucial roles of FHL1 in myogenic differentiation and maintenance (24). In line with their research on primary chicken myoblasts, this study showed that FHL1 knockdown with siRNA was capable of suppressing the expression of MyoD, MyoG, MEF2C, and MyHC during myogenic differentiation and significantly impaired myotube formation in C2C12 myoblasts (Fig. 3 and Fig. 4). Recently, the roles of FHL1 in skeletal muscle were investigated in genetically modified mice models. The loss of FHL1 caused an aberrant muscle fiber structure with reduced strength and activated autophagy in skeletal muscle (21, 22). Therefore, most of the previous studies indicated that FHL1 plays a vital role in skeletal muscle myogenesis and maintenance. Interestingly, PA drastically inhibited the expression of myogenic factors and myogenic differentiation in C2C12 myoblasts that accompanied the suppression of FHL1 expression (Fig. 1). As the transfection of FHL1 siRNA severely inhibited the expression of myogenic factors and myotube formation in myoblasts (Fig. 3 and Fig. 4), the reduction of FHL1 is one of the critical contributing factors to impaired myogenesis resulting from PA treatment.

Accumulating evidence indicates that miRNAs have a significant impact on myogenesis and muscle homeostasis (11, 25). Nevertheless, there are no reports focused on the role of miR-183-5p in myogenesis. Hsa-miR-183-5p is a member of

the miR-183 family gene cluster, and it plays multiple roles in a wide range of physiological and pathological processes, such as cell proliferation, apoptosis, metabolism, and immunity (26). The present study for the first time revealed the novel roles of miR-183-5p on FHL1 expression and myogenic differentiation in C2C12 myoblasts. In particular, miR-183-5p was upregulated significantly in PA-treated myoblasts and suppressed the expression of FHL1 through direct binding to 3′UTR on FHL1 mRNA. The lack of miR-183-5p binding sites on the 3′UTRs of myogenic transcription factors means that the inhibition of myogenic differentiation observed in miR-183-5p transfected cells is attributed primarily to the suppression of FHL1 by miR-183-5p. Thus, the upregulation of miR-183-5p inhibits myogenic differentiation by reducing FHL1 expression and might be a detrimental factor for myogenesis and muscle mass maintenance.

The underlying regulatory mechanism through which PA upregulates miR-183-5p expression in myoblasts remains open questions. On the other hand, based on *in silico* analysis, the promoter regions of miR-183-5p contain possible binding sites for various transcription factors associated with adipogenesis and obesity, such as PPARγ and C/EBPα. PPARγ is a crucial transcription factor in mammalian adipogenesis and is closely linked to obesity (27). PPARγ is also coactivated with C/EBPα, an adipogenic transcription factor that is activated in high-fat diet-fed obese mice (28). Therefore, the activation of PPARγ and C/EBPα may contribute to the induction of miR-183-5p in obesity. Interestingly, miR-183-5p was upregulated during mouse adipogenesis by the activation of PPARγ (29), and its levels were increased in the liver of high-fat diet-fed mice (30). Although its upregulation has been shown in non-muscle tissues, miR-183-5p was suggested to play a role as a plasma-driven exosomal miRNA in intercellular communication (31, 32). Further studies on the transcriptional factors involved and how they regulate miR-183-5p will be investigated in a future study.

In conclusion, this study investigated the implications of miR-183-5p induced by PA in myogenic differentiation. MiR-183-5p was found to downregulate FHL1 expression by targeting the 3′UTR of FHL1. Furthermore, overexpression of miR-183-5p impeded myoblast differentiation and myotube formation. Thus, this study reveals the inhibitory effect of miR-183-5p on myogenic differentiation by suppressing FHL1 expression and suggests a novel mechanism for myogenesis regulated by miRNA in a model of obesity.

MATERIALS AND METHODS

See supplementary information for Material and Methods.

ACKNOWLEDGEMENTS

This study was supported by the National Research Foundation of Korea (NRF) funded by the Korean government (Grant nos. NRF-2017M2B2A4049415 and NRF-2019R1F1A1040858) and by the Outstanding Researchers Program of Dongguk University (Grant no. K-2018-G0002-00016).

CONFLICTS OF INTEREST

The authors have no conflicting interests.

REFERENCES

1. Chal J and Pourquie O (2017) Making muscle: skeletal myogenesis in vivo and in vitro. Development 144, 2104-2122
2. Asfour HA, Allouh MZ and Said RS (2018) Myogenic regulatory factors: The orchestrators of myogenesis after 30 years of discovery. Exp Biol Med (Maywood) 243, 118-128
3. Benarroch L, Bonne G, Rivier F and Hamroun D (2019) The 2020 version of the gene table of neuromuscular disorders (nuclear genome). Neuromuscul Disord 29, 980-1018
4. Akhmedov D and Berdeaux R (2013) The effects of obesity on skeletal muscle regeneration. Front Physiol 4, 371
5. Chang YC, Liu HW, Chen YT, Chen YA, Chen YJ and Chang SJ (2018) Resveratrol protects muscle cells against palmitate-induced cellular senescence and insulin resistance through ameliorating autophagic flux. J Food Drug Anal 26, 1066-1074
6. Zhang G, Chen X, Lin L, Wen C and Rao S (2012) [Effects of fatty acids on proliferation and differentiation of myoblast]. Wei Sheng Yan Jiu 41, 883-888
7. Saini A, Sharples AP, Al-Shanti N and Stewart CE (2017) Omega-3 fatty acid EPA improves regenerative capacity of mouse skeletal muscle cells exposed to saturated fat and inflammation. Biogerontology 18, 109-129
8. Xu D, Jiang Z, Sun Z et al (2019) Mitochondrial dysfunction and inhibition of myoblast differentiation in mice with high-fat-diet-induced pre-diabetes. J Cell Physiol 234, 7510-7523
9. Krol J, Loedige I and Filipowicz W (2010) The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 11, 597-610
10. Saliminejad K, Khorram Khorshid HR, Soleymani Fard S and Ghaffari SH (2019) An overview of microRNAs: Biology, functions, therapeutics, and analysis methods. J Cell Physiol 234, 5451-5465
11. Mok GF, Lozano-Velasco E and Münsterberg A (2017) microRNAs in skeletal muscle development. Semin Cell Dev Biol 72, 67-76
12. Ji C and Guo X (2019) The clinical potential of circulating microRNAs in obesity. Nat Rev Endocrinol 15, 731-743
13. Ortiz-Dosal A, Rodil-García P and Salazar-Olivo LA (2019) Circulating microRNAs in human obesity: a systematic review. Biomarkers 24, 499-509
14. Kadrmas JL and Beckerle MC (2004) The LIM domain: from the cytoskeleton to the nucleus. Nat Rev Mol Cell Biol 5, 920-931

15. Wei X and Zhang H (2020) Four and a half LIM domains protein 1 can be as a double-edged sword in cancer progression. Cancer Biol Med 17, 270-281
16. Windpassinger C, Schoser B, Straub V et al (2008) An X-linked myopathy with postural muscle atrophy and generalized hypertrophy, termed XMPMA, is caused by mutations in FHL1. Am J Hum Genet 82, 88-99
17. Chen DH, Raskind WH, Parson WW et al (2010) A novel mutation in FHL1 in a family with X-linked scapuloperoneal myopathy: phenotypic spectrum and structural study of FHL1 mutations. J Neurol Sci 296, 22-29
18. Gueneau L, Bertrand AT, Jais JP et al (2009) Mutations of the FHL1 gene cause Emery-Dreifuss muscular dystrophy. Am J Hum Genet 85, 338-353
19. Cowling BS, McGrath MJ, Nguyen MA et al (2008) Identification of FHL1 as a regulator of skeletal muscle mass: implications for human myopathy. J Cell Biol 183, 1033-1048
20. Lee JY, Chien IC, Lin WY et al (2012) Fhl1 as a downstream target of Wnt signaling to promote myogenesis of C2C12 cells. Mol Cell Biochem 365, 251-262
21. Domenighetti AA, Chu PH, Wu T et al (2014) Loss of FHL1 induces an age-dependent skeletal muscle myopathy associated with myofibrillar and intermyofibrillar disorganization in mice. Hum Mol Genet 23, 209-225
22. Ding J, Cong YF, Liu B, Miao J and Wang L (2018) Aberrant protein turn-over associated with myofibrillar disorganization in FHL1 knockout mice. Front Genet 9, 273
23. Abreu P, Leal-Cardoso JH, Ceccatto VM and Hirabara SM (2017) Regulation of muscle plasticity and trophism by fatty acids: A short review. Rev Assoc Med Bras (1992) 63, 148-155
24. Han S, Cui C, He H et al (2019) FHL1 regulates myoblast differentiation and autophagy through its interaction with LC3. J Cell Physiol 235, 4667-4678
25. Zhao Y, Chen M, Lian D et al (2019) Non-coding RNA regulates the myogenesis of skeletal muscle satellite cells, injury repair and diseases. Cells 8, 988
26. Dambal S, Shah M, Mihelich B and Nonn L (2015) The microRNA-183 cluster: the family that plays together stays together. Nucleic Acids Res 43, 7173-7188
27. Shao X, Wang M, Wei X et al (2016) Peroxisome proliferator-activated receptor-gamma: master regulator of adipogenesis and obesity. Curr Stem Cell Res Ther 11, 282-289
28. Lefterova MI, Zhang Y, Steger DJ et al (2008) PPARgamma and C/EBP factors orchestrate adipocyte biology via adjacent binding on a genome-wide scale. Genes Dev 22, 2941-2952
29. John E, Wienecke-Baldacchino A, Liivrand M, Heinaniemi M, Carlberg C and Sinkkonen L (2012) Dataset integration identifies transcriptional regulation of microRNA genes by PPARgamma in differentiating mouse 3T3-L1 adipocytes. Nucleic Acids Res 40, 4446-4460
30. Yang WM, Min KH and Lee W (2016) MicroRNA expression analysis in the liver of high fat diet-induced obese mice. Data Brief 9, 1155-1159
31. Zhao X, Jia Y, Chen H, Yao H and Guo W (2019) Plasma-derived exosomal miR-183 associates with protein kinase activity and may serve as a novel predictive biomarker of myocardial ischemic injury. Exp Ther Med 18, 179-187
32. Feng Y, Hang W, Sang Z et al (2019) Identification of exosomal and nonexosomal microRNAs associated with the drug resistance of ovarian cancer. Mol Med Rep 19, 3376-3392