Acta Biochim Biophys Sin, 2016, 48(9), 833–839
doi: 10.1093/abbs/gmw077
Advance Access Publication Date: 25 August 2016
Original Article

Original Article

miR-378 attenuates muscle regeneration by delaying satellite cell activation and differentiation in mice

Ping Zeng[1,†], Wanhong Han[2,†], Changyin Li[2], Hu Li[2], Dahai Zhu[2], Yong Zhang[2,*], and Xiaohong Liu[1,*]

[1]Division of Geriatrics, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing 100730, China, and [2]The State Key Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and School of Basic Medicine, Peking Union Medical College, Beijing 100005, China

[†]These authors contributed equally to this work.
*Correspondence address. Tel: +86-10-69154006; Fax: +86-10-69154063; E-mail: xhliu41@medmail.com.cn (X.L.)/
Tel: +86-10-65105081; Fax: +86-10-65105083; E-mail: yongzhang@ibms.pumc.edu.cn (Y.Z.)

Received 5 April 2016; Accepted 16 May 2016

Abstract

Skeletal muscle mass and homeostasis during postnatal muscle development and regeneration largely depend on adult muscle stem cells (satellite cells). We recently showed that global overexpression of miR-378 significantly reduced skeletal muscle mass in mice. In the current study, we used miR-378 transgenic (Tg) mice to assess the *in vivo* functional effects of miR-378 on skeletal muscle growth and regeneration. Cross-sectional analysis of skeletal muscle tissues showed that the number and size of myofibers were significantly lower in miR-378 Tg mice than in wild-type mice. Attenuated cardiotoxin-induced muscle regeneration in miR-378 Tg mice was found to be associated with delayed satellite cell activation and differentiation. Mechanistically, miR-378 was found to directly target *Igf1r* in muscle cells both *in vitro* and *in vivo*. These miR-378 Tg mice may provide a model for investigating the physiological and pathological roles of skeletal muscle in muscle-associated diseases in humans, particularly in sarcopenia.

Key words: miR-378, muscle regeneration, satellite cell activation, differentiation, *Igf1r*

Introduction

Skeletal muscle accounts for 40% of body weight and is physiologically significant, acting not only as a supportive organ but also as a metabolic and endocrine organ during development [1–4]. Muscle mass and quality are physiologically important in maintaining whole body homeostasis, and muscle loss associated with pathological conditions, including cancer, chronic heart disease, liver cirrhosis, Crohn's disease, rheumatoid arthritis, tuberculosis, and age-related sarcopenia, can impair activities of daily living and significantly reduce quality of life [5,6]. Understanding the mechanisms underlying skeletal muscle atrophy in these conditions may assist in the design of therapeutic strategies to prevent and treat skeletal muscle loss associated with these diseases.

Postnatal muscle growth and maintenance of muscle homeostasis largely rely on adult muscle stem cells, also known as satellite cells [7–9]. In resting muscle, satellite cells are mostly quiescent; however, in response to stress or injury, these cells are activated, proliferate, and undergo differentiation to repair damaged muscle or self-renewal to replenish the quiescent cell pool [10–12]. Reduced satellite cell function has been observed in patients with chronic diseases, including cancer-induced cachexia and diabetes [13,14]. Moreover, reduced muscle stem cell function may be a potentially important

mechanism in the pathogenic development of age-related sarcopenia [15,16].

Several signaling pathways have been implicated in the regulation of satellite cell-mediated muscle growth and homeostasis, including Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling [17], the p38α and p38β mitogen-activated kinase pathway [18] and the insulin-like growth factor I receptor (IGF1R)/phosphatidylinositol 3-kinase (PI3K) pathway [19–21]. Recently, miRNAs have been found to be involved in muscle development and regeneration [22,23]. A subset of miRNAs that is either specifically expressed or predominantly enriched in skeletal and/or cardiac muscle has been identified, including miRNAs-1, -133, -206, -208a, -208b, -499, and -486 [24]. miRs-1, -133, and -206 are up-regulated during myogenic differentiation and can be inhibited by inhibitors of the PI3K/AKT pathway [25]. In addition, miR-431 was found to enhance muscle generation by targeting Pax7 in satellite cells primed for differentiation during muscle regeneration [26].

miR-378, which is embedded within the first intron of the gene encoding peroxisome proliferator-activated receptor γ coactivator 1β (*Ppargc1b* or *Pgc-1β*), is preferentially expressed in skeletal muscle, cardiac muscle, and brown adipose tissue [27,28]. The physiological mechanisms by which miR-378 regulates muscle development and regeneration *in vivo* remain to be determined. We previously reported that skeletal muscle mass was significantly reduced in transgenic mice globally overexpressing miR-378 under the control of the *β-actin* promoter (pCAGGS) [29]. As an extension of that study, in the present work we evaluated the *in vivo* functions of miR-378 in miR-378 Tg mice, and found that miR-378 regulates skeletal development and regeneration by targeting the gene encoding insulin-like growth factor 1 receptor (*Igf1r*).

Materials and Methods

Animal care and tissue collection

All animal experiment procedures were approved by the Animal Ethics Committee of Peking Union Medical College (Beijing, China). Mice were housed in the animal facility and had free access to water and standard rodent chow. miR-378 Tg mice (two transgenic lines, C and D lines) were generated as described previously [29].

Muscle injury and regeneration

To induce muscle injury, 8-week-old mice were anesthetized by intraperitoneal injection of ketamine (10 mg/kg) and xylazine (1 mg/kg), followed by injection of 20 μl of 10 μM cardiotoxin (CTX; Sigma, St Louis, USA) in phosphate-buffered saline (PBS) into the mid-belly of the right tibialis anterior (TA) muscle. As an internal control, 20 μl PBS was injected into the left TA muscle of each mouse. Muscles were harvested 1.5, 3.5, 5, and 7 days after injection to assess the completion of regeneration and repair.

Cell culture and cell transfection

C2C12 and HEK293 cells were cultured in growth medium consisting of DMEM (Gibco, Carlsbad, USA) supplemented with 4.5 g/l glucose, 10% FBS, 1% antibiotic/antimycotic, and 1% gentamycin at 37°C in a 5% CO_2 atmosphere. C2C12 and HEK293 cells were transfected with miR-378 mimics (RiboBio, Guangzhou, China) using Lipofectamine 2000 (Invitrogen, Carlsbad, USA).

Western blot analysis

Muscle tissues or C2C12 cells were lysed in a buffer containing 50 mM Tris (pH 7.5), 150 mM NaCl, 0.5% Nonidet P-40, and protease and phosphatase inhibitors. The protein lysates were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to polyvinylidene fluoride membranes, and immunoblotted with primary antibodies against IGF1R (Cell Signaling, Beverly, USA) and GAPDH (Millipore, Billerica, USA). The membranes were washed for 30 min, incubated with horseradish peroxidase-conjugated secondary antibodies (Zhongshanjinqiao Corporation, Beijing, China) for 1 h at room temperature, and washed again for 30 min. Each membrane was then placed into ECL detection solution (Thermo Scientific, Waltham, USA), incubated for 1 min at room temperature, and exposed to X-ray film.

Immunohistochemistry

Frozen tissue sections (10-μm thick) were fixed in 4% paraformaldehyde and stained with antibody to laminin (Sigma), Pax7 (Developmental Studies Hybridoma Bank, Iowa City, USA), or MyoD (Santa Cruz Biotechnology, Santa Cruz, USA), as described previously [26]. Cross-sectional areas (CSAs) were measured using NIH Image J software (http://rsb.info.nih.gov/ij).

Real-time reverse transcriptase polymerase chain reaction

Total RNA was extracted from skeletal muscle tissue samples with TRIzol reagent (Invitrogen). mRNA expression was analyzed with Fast EvaGreen qPCR Master Mix (BioRad, Hercules, USA) and normalized to *GAPDH*. Primers for PCR were as follows: *MyoD*-F: 5′-CAA CGCCATCCGCTACAT-3′; *MyoD*-R: 5′-GGTCTGGGTTCCCTGTT CT-3′; *eMHC*-F: 5′-TCTAGCCGGATGGTGGTCC-3′; *eMHC*-R: 5′-GAATTGTCAGGAGCCACGAAA-3′; *GAPDH*-F: 5′-AATGT GTCCGTCGTGGATCTG-3′; *GAPDH*-R: 5′-TAGCCCAAGAT GCCCTTCAGT-3′.

Target-gene prediction and luciferase-reporter assays

miR-378 targets were predicted with TargetScan. To construct the 3′-UTR-luciferase reporter, the multiple cloning site of the pGL3-Control vector was removed and placed downstream of the luciferase gene. The 3′-UTRs of mouse *Igf1r* were amplified by PCR and cloned into the pGL3-Control vector, resulting in *Igf1r*-3′-UTR constructs. Empty pGL3-Control vector was used as a negative control. Each firefly luciferase construct was co-transfected with a plasmid encoding a Renilla luciferase construct as a transfection control. The results were expressed as firefly luciferase activity relative to Renilla luciferase activity.

Grip strength assay

Grip strength was measured as described previously [30,31]. Briefly, limb grip strength was measured as tension force using a computerized force transducer (Grip Strength Meter, Bioseb, Pinellas Park, USA). Each animal was subject to three trials, with three measurements per trial and a rest period of a few minutes between trials. The average tension force corrected by total body weight was calculated for each group of mice.

Statistical analysis

Values were presented as the mean ± standard error of the mean (SEM), and compared by Student's *t*-tests. $P < 0.05$ was considered statistically significant.

Results

miR-378 transgenic mice have fewer and smaller myofibers

We recently reported that skeletal muscle mass was significantly reduced in transgenic mice globally overexpressing miR-378 compared with that in their wild-type (Wt) littermates [29]. To further evaluate the mechanism by which miR-378 regulates muscle development and regeneration *in vivo*, we performed cross-sectional analyzes of the TA and soleus muscles from miR-378 Tg and Wt mice. Muscle histology as well as hematoxylin and eosin (H&E) staining showed no differences in muscular architecture (data not shown). To compare the number and size of myofibers in miR-378 Tg and Wt mice, cryosections of their soleus muscles were stained with anti-laminin antibody (**Fig. 1A**) and analyzed with Image J software. Both the number (**Fig. 1B**) and size (**Fig. 1C**) of fibers were significantly reduced in miR-378 Tg mice. Consistent with the reductions in muscle mass and fiber size, muscle strength was markedly lower in miR-378 Tg than in Wt mice (**Fig. 1D**). As the two transgenic lines (C and D lines) of miR-378 Tg mice have a similar phenotype, only D line was further characterized in detail.

miR-378 Tg mice exhibit delayed muscle regeneration resulting from attenuated satellite cell differentiation

As skeletal muscle loss has been reported because of declined satellite cell function [12], muscle loss in miR-378 Tg mice may be resulted from miR-378-mediated abnormalities of satellite cell functions. This possibility was addressed experimentally by evaluating muscle injury and regeneration in miR-378 Tg and Wt littermates following injection of CTX. During muscle regeneration, satellite cells are activated, subsequently expanding and differentiating to fuse with each other and then repair damaged muscle. Therefore, satellite cell functions during muscle regeneration could be evaluated by measuring the size of newly-formed (regenerated) myofibers, as characterized by centralized nuclei. Cryosections of CTX-damaged TA muscles 5 days (**Fig. 2A**) and 7 days (**Fig. 2D**) after CTX injection were immunostained with anti-laminin antibody and with DAPI to visualize nuclei. The regenerated myofibers, as determined with Image J software, were significantly smaller in miR-378 Tg than in Wt mice both 5 days (**Fig. 2B**) and 7 days (**Fig. 2E**) after CTX-induced injury, indicating that muscle regeneration was significantly slower in miR-378 Tg than in Wt mice. Furthermore, the myogenic differentiation marker, embryonic myosin heavy chain (*eMyHC*), was highly expressed in the damaged TA muscle of miR-378 Tg mice 5 days after CTX-induced injury (**Fig. 2C**), and was even higher 7 days after CTX-induced injury (**Fig. 2F**) than in Wt mice, suggesting that the slower formation of neonatal myofibers in miR-378 Tg than in Wt mice may be attributed to delayed satellite cell differentiation in the former. The observation was further corroborated by eMyHC immunostaining on the cryosections of the TA muscle 7 days post-CTX injury in miR-378 transgenic mice and wild-type littermates (**Fig. 2G**). In miR-378 Tg mice, the number of eMyHC positive myofibers in the TA muscle 7 days post-CTX injury was significantly more than that in wild-type littermates (**Fig. 2H**). These findings indicate that delayed muscle regeneration in miR-378 Tg mice may be due, at least in part, to attenuated satellite cell differentiation.

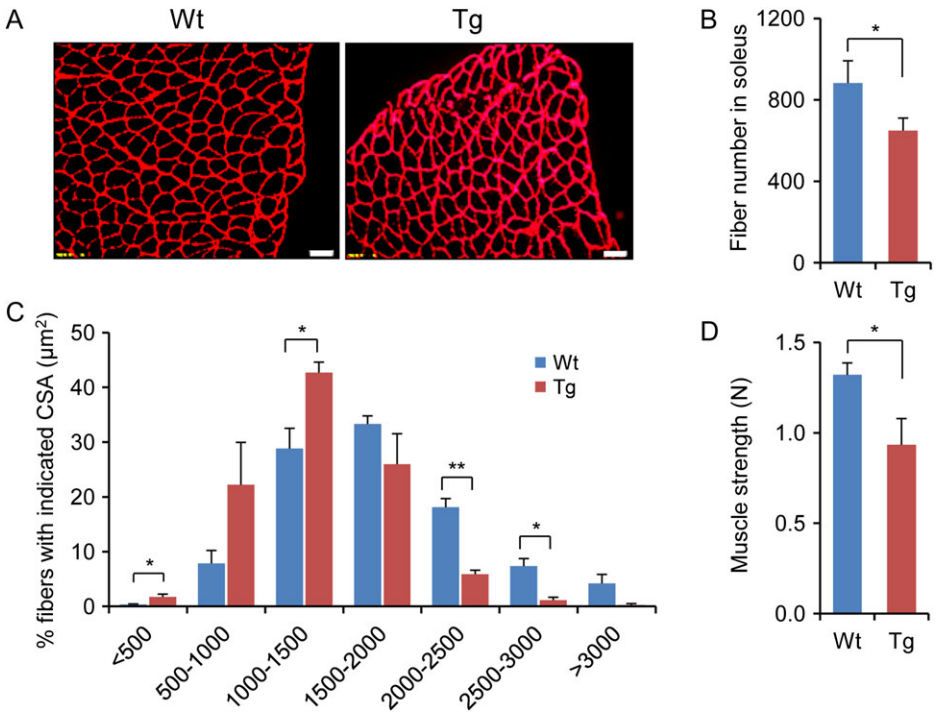

Figure 1. The number and size of myofibers are reduced in miR-378 transgenic mice (A) Immunostaining of cryosections of soleus muscle from miR-378 transgenic (Tg) and wild-type (Wt) mice with anti-laminin antibody. The images are representative of five pairs of 2-month-old Tg and Wt mice. Scale bars, 50 μm. (B) Number of myofibers in soleus muscle based on laminin-stained sections in panel A. (C) Cross-sectional area (CSA) of myofibers based on laminin-stained sections in panel A. (D) Grip strength of miR-378 Tg and Wt mice ($n = 5$–8). *$P < 0.05$, **$P < 0.01$.

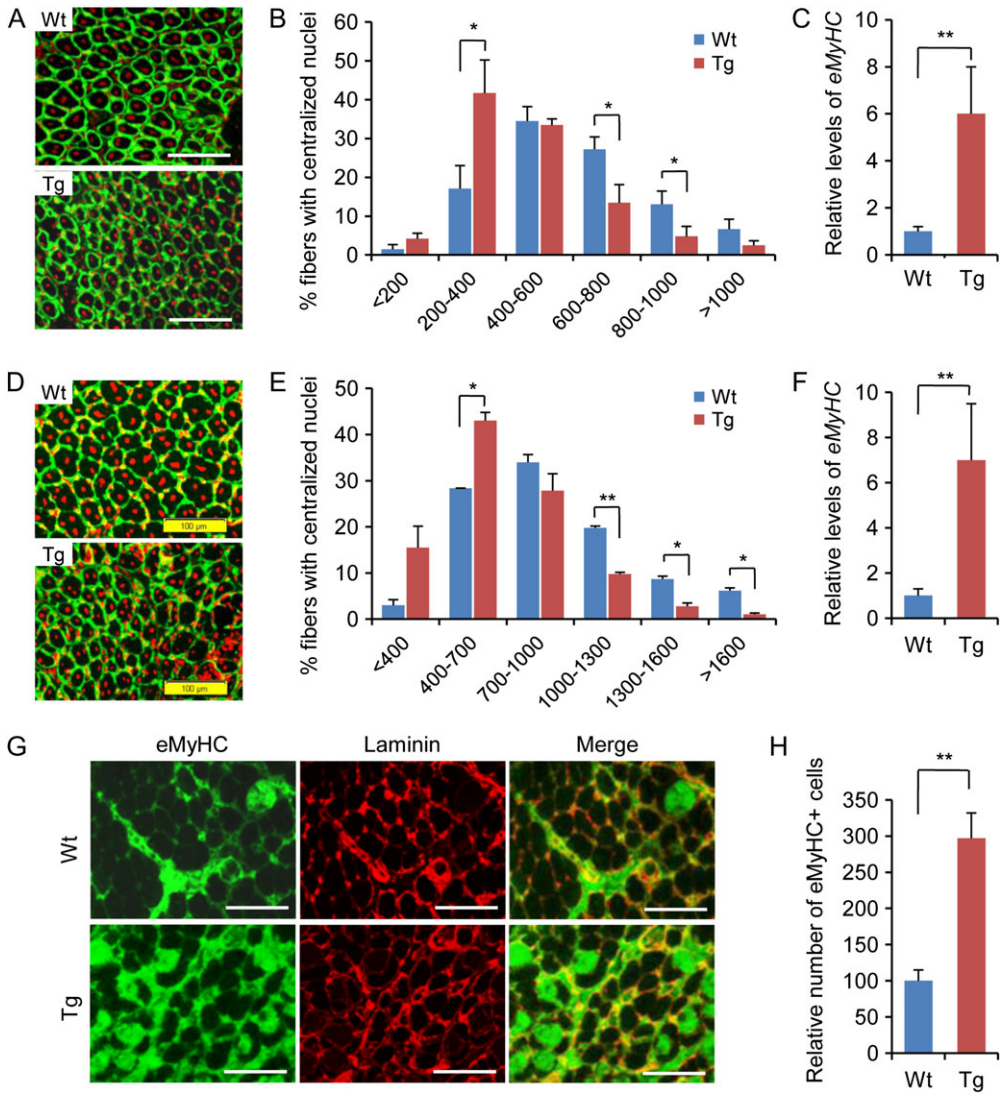

Figure 2. miR-378 Tg mice exhibit delayed muscle regeneration due to attenuated satellite cell differentiation (A,D) Representative images of laminin-stained (green) cryosections of damaged TA muscle 5 (A) and 7 (D) days after injection of CTX. DAPI staining (red) indicates nuclei. Scale bars, 100 μm. (B, E) Cross-sectional areas (μm^2) of regenerated myofibers with centralized nuclei, based on laminin-stained sections in panels A (B) and D (E). (C and F) Expression of *eMyHC* mRNA in damaged TA muscle 5 (C) and 7 (F) days after CTX injection, as determined by Real-time reverse transcriptase polymerase chain reaction (qRT-PCR). (G) eMyHC immunostaining on the cryosections of the TA muscle 7 days post-CTX injury in miR-378 Tg mice and Wt littermates. Laminin staining (red) indicates basal lamina of myofibers. Scale bars, 100 μm. (H) eMyHC positive myofibers were calculated based on eMyHC-stained sections presented in (G). The data are from five pairs of miR-378 Tg and Wt littermates. $*P < 0.05$, $**P < 0.01$.

Delayed activation of satellite cells in miR-378 Tg mice during muscle regeneration

Satellite cell-mediated muscle regeneration involves a cascade of satellite cell activation, proliferation, and differentiation. We therefore assessed the ability of miR-378 to regulate satellite cell activation and proliferation. During the process of muscle regeneration following CTX injection, satellite cells become fully activated, as shown by the expressions of Pax7 and MyoD, after 1.5 days, with the growth of satellite cells peaking after 3.5 days. Cryosections of damaged TA muscle from miR-378 Tg mice and Wt controls were immunostained with antibody to the satellite cell activation marker MyoD (**Fig. 3A**). Fewer $MyoD^+$ cells were present (**Fig. 3B**), and the level of expression of *MyoD* was lower (**Fig. 3C**) in Tg mice than in Wt mice, suggesting that the activation of satellite cells was delayed in the former. As proliferating satellite cells are doubly positive for Pax7 and MyoD, cryosections of damaged TA muscle from Tg mice and Wt mice 3.5 days after injury were stained with anti-Pax7 and anti-MyoD antibodies, and then the numbers of $Pax7^+/MyoD^+$ cells were counted (**Fig. 3D**). The numbers of $Pax7^+$ (**Fig. 3E**), $MyoD^+$ (**Fig. 3F**) and $Pax7^+/MyoD^+$ (**Fig. 3G**) cells did not differ significantly between groups of miR-378 Tg mice and Wt mice, suggesting that miR-378 has little effect on satellite cell proliferation. Collectively, our data indicate that miR-378 attenuates muscle regeneration by delaying satellite cell activation and differentiation.

miR-378 directly targets Igf1r in muscle cells both *in vitro* and *in vivo*

To understand the molecular mechanisms underlining miR-378-mediated muscle loss and attenuation of muscle regeneration,

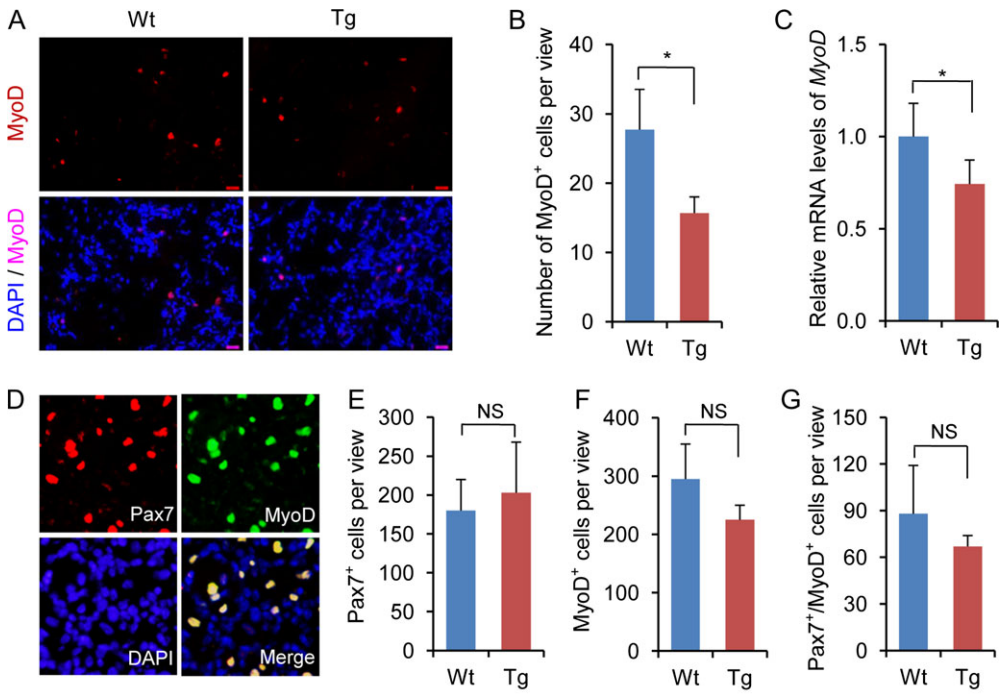

Figure 3. Delayed activation of satellite cells in miR-378 Tg mice during muscle regeneration (A) Representative images of MyoD-stained (red) sections of damaged TA muscle of miR-378 Tg and Wt mice 1.5 days after CTX injection ($n = 5$ each). DAPI staining (blue) indicates nuclei. Scale bars, 20 μm. (B) Number of MyoD positive cells based on MyoD-stained sections in A. (C) Expression of *MyoD* mRNA, normalized to *GAPDH*, in damaged TA muscle 1.5 days after CTX injection, as determined by qRT-PCR. Data are presented relative to Wt controls, which were set at 1. (D) Representative images of damaged TA muscle from miR-378 Tg and Wt mice ($n = 5$ each) 3.5 days after CTX injection after staining with anti-Pax7 (red) and anti-MyoD (green) antibodies. DAPI staining (blue) indicates nuclei. (E–G) Numbers of Pax7 positive (E), MyoD positive (F) and double positive cells (G), calculated based on immunostained TA sections in panel D. *$P < 0.05$; NS, not significant.

we attempted to identify the targets of miR-378 in muscle cells. Interestingly, by searching for miR-378 targets with TargetScan, we found that the 3′-UTR of insulin-like growth factor 1 receptor (*Igf1r*) mRNA contains a miR-378 binding site (**Fig. 4A**). The signaling pathways involving IGF1 and IGF1R are important in regulating myogenic differentiation and muscle mass during muscle development and regeneration [21,32]. To confirm that *Igf1r* is a target of miR-378, HEK-293 cells were transfected with miR-378 and a luciferase reporter construct containing a partial sequence of the *Igf1r* 3′-UTR encompassing the miR-378 binding site. It was found that luciferase activity was repressed (**Fig. 4B**). In addition, the level of IGF1R protein in C2C12 cells transfected with miR-378 mimics was significantly reduced (**Fig. 4C**). Consistent with these *in vitro* data, the IGF1R protein levels were found to be lower in skeletal muscle from miR-378 Tg mice than in Wt mice (**Fig. 4D**). These findings indicate that miR-378 directly targets *Igf1r* both *in vitro* and *in vivo*.

Discussion

Muscle loss is frequently observed in chronic diseases, including cancer, chronic heart disease, liver cirrhosis, Crohn's disease, rheumatoid arthritis, and tuberculosis, as well as during age-related sarcopenia [6]. As prevention of muscle loss can improve patients' functional status and quality of life, it is of great importance to determine the mechanisms underlying muscle loss. This study showed that miR-378 transgenic mice had a muscle loss phenotype and defective muscle regeneration due to an attenuated IGF1R signaling pathway.

Our experimental data demonstrate that miR-378 directly targets *Igf1r* both *in vitro* and *in vivo*. IGF1R-mediated signaling has been shown to enhance myogenic differentiation [33–35]. Consistent with the reduced IGF1R signaling in skeletal muscle of miR-378 Tg mice, it was found that satellite cell differentiation was delayed and muscle regeneration was attenuated in these mice, suggesting that miR-378 may functionally inhibit muscle cell differentiation by targeting *Igf1r*. These findings, however, conflict with previously published data related to miR-378-mediated muscle cell differentiation [36,37]. For example, miR-378 was reported to accelerate C2C12 cell differentiation by targeting the myogenic differentiation inhibitor *MyoR* [36], and to promote C2C12 cell differentiation by targeting *BMP4* [37]. These discrepancies may be due to differences in the *in vivo* and *in vitro* activities of miR-378. To the best of our knowledge, this study is the first to show that miR-378 regulates muscle cell differentiation *in vivo*. In addition, we previously found that miR-378 regulates metabolic homeostasis in skeletal muscle by targeting the Akt1/FoxO1/PEPCK pathway [29]. Therefore, it is very likely that the modified metabolic niche in skeletal myofibers mediated by miR-378 greatly influences satellite cell function. Therefore, the miRNA-target pairs may function differently, depending on biological context (niche). The development of satellite cell specific miR-378 transgenic mice may help clarify the physiological function of miR-378 in muscle cell differentiation *in vivo*.

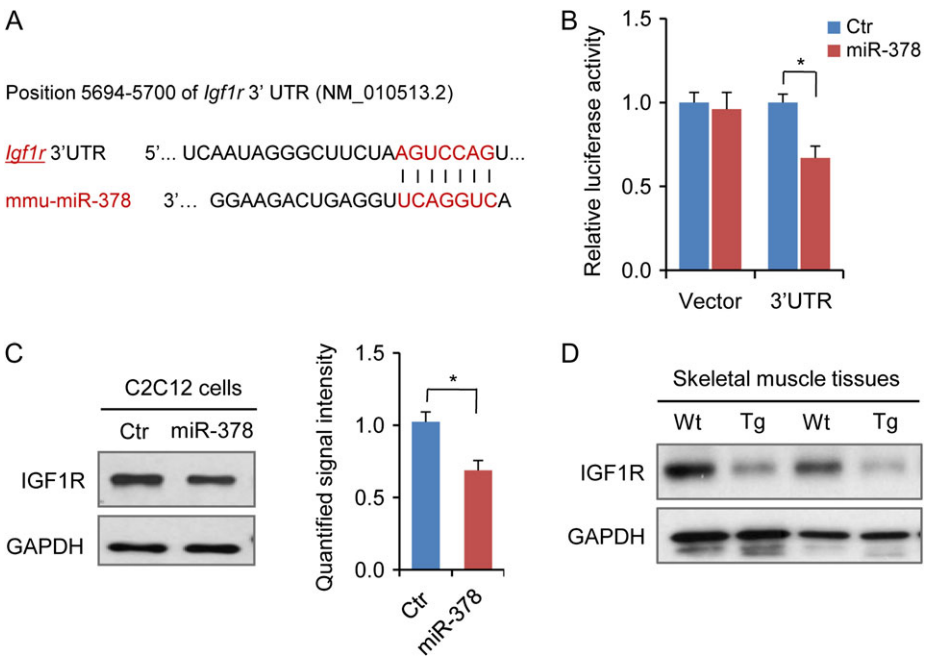

Figure 4. miR-378 directly targets *Igf1r* in muscle cells *in vitro* and *in vivo* (A) miR-378 target sequence alignment in the *Igf1r* 3′-UTR predicted with TargetScan. The nucleotide coordinate of *Igf1r* was based on the mouse RefSeq (NM_010513.2). miR-378 seed matches are indicated in red. (B) Luciferase assays measuring the effect of transfection of miR-378 (mimics) into HEK 293 cells; transfection of non-specific scrambled oligonucleotides served as control (Ctr). The pGL3-control empty vector was the negative control. 3′-UTR indicated insertion of a perfectly complementary target of miR-378 downstream of the luciferase gene in the pGL3-control vector. Values are the mean ± SEM of three measurements. (C) C2C12 cells were transfected with miR-378 mimics, and IGF1R protein levels were determined by western blot analysis; GAPDH served as a loading control. The signal intensities on the blots (left panel) were quantified by Image J software (right panel). Values are the mean ± SEM. of three measurements. (D) IGF1R protein levels in skeletal muscle tissues of miR-378 Tg and Wt littermates, as measured by western blot analysis; GAPDH served as a loading control. $^*P < 0.05$.

In addition to its involvement in myogenic differentiation, IGF1R signaling plays important roles in muscle growth and hypertrophy [38]. The IGF1R/Akt pathway is one of the main signaling pathways controlling protein turnover in skeletal muscle. IGF-1 has been shown to activate the phosphatidylinositol 3-kinase (PI3K)-Akt pathway, with inhibition of this pathway enhancing proteolysis and the expression of atrogin-1 [39]. The induction of atrogin-1 as well as of murf1, another muscle-specific E3 similar to atrogin-1, plays an important role in the loss of muscle protein [32]. Consistent with the attenuated IGF1R signaling observed in skeletal muscle of miR-378 Tg mice, muscle atrophy, as evidenced by reduced myofiber size and number, was evident. Taken together, these findings indicate that miR-378 induces skeletal muscle loss by targeting *Igf1r*.

In conclusion, the present findings demonstrate that miR-378 Tg mice are characterized by skeletal muscle loss and dysfunction in muscle regeneration, with miR-378 regulating muscle loss and regeneration by targeting *Igf1r*. These miR-378 transgenic mice may constitute a model for investigating the molecular mechanisms of skeletal muscle disease, especially sarcopenia.

Funding

This work was supported by the grants from the National Basic Research Program of China (No. 2015CB943103), the National Natural Science Foundation of China (Nos. 31271470 and 91540206) and the Youth Foundation of Peking Union Medical College (No. 3332015089).

References

1. Garriga J, Fernandez-Sola J, Adanero E, Urbano-Marquez A, Cusso R. Metabolic effects of ethanol on primary cell cultures of rat skeletal muscle. *Alcohol* 2005, 35: 75–82.
2. Peppa M, Koliaki C, Nikolopoulos P, Raptis SA. Skeletal muscle insulin resistance in endocrine disease. *J Biomed Biotechnol* 2010, 2010: 527850.
3. Aguer C, Harper ME. Skeletal muscle mitochondrial energetics in obesity and type 2 diabetes mellitus: endocrine aspects. *Best Pract Res Clin Endoc Metab* 2012, 26: 805–819.
4. Keipert S, Ost M, Johann K, Imber F, Jastroch M, van Schothorst EM, Keijer J, *et al*. Skeletal muscle mitochondrial uncoupling drives endocrine cross-talk through the induction of FGF21 as a myokine. *Am J Physiol Endocrinol Metab* 2014, 306: E469–E482.
5. Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *J Am Geriatr Soc* 2002, 50: 889–896.
6. Drescher C, Konishi M, Ebner N, Springer J. Loss of muscle mass: current developments in cachexia and sarcopenia focused on biomarkers and treatment. *Int J Cardiol* 2015, 202: 766–772.
7. Cerletti M, Shadrach JL, Jurga S, Sherwood R, Wagers AJ. Regulation and function of skeletal muscle stem cells. *Cold Spring Harb Symp Quant Biol* 2008, 73: 317–322.
8. Biressi S, Rando TA. Heterogeneity in the muscle satellite cell population. *Semin Cell Dev Biol* 2010, 21: 845–854.
9. Pannerec A, Marazzi G, Sassoon D. Stem cells in the hood: the skeletal muscle niche. *Trends Mol Med* 2012, 18: 599–606.
10. Lepper C, Partridge TA, Fan CM. An absolute requirement for Pax7-positive satellite cells in acute injury-induced skeletal muscle regeneration. *Development* 2011, 138: 3639–3646.

11. Murphy MM, Lawson JA, Mathew SJ, Hutcheson DA, Kardon G. Satellite cells, connective tissue fibroblasts and their interactions are crucial for muscle regeneration. *Development* 2011, 138: 3625–3637.
12. Sousa-Victor P, Gutarra S, Garcia-Prat L, Rodriguez-Ubreva J, Ortet L, Ruiz-Bonilla V, Jardi M, *et al*. Geriatric muscle stem cells switch reversible quiescence into senescence. *Nature* 2014, 506: 316–321.
13. Yoshida T, Delafontaine P. Mechanisms of cachexia in chronic disease states. *Am J Med Sci* 2015, 350: 250–256.
14. Krause MP, Al-Sajee D, D'Souza DM, Rebalka IA, Moradi J, Riddell MC, Hawke TJ. Impaired macrophage and satellite cell infiltration occurs in a muscle-specific fashion following injury in diabetic skeletal muscle. *PLoS One* 2013, 8: e70971.
15. Jang YC, Sinha M, Cerletti M, Dall'Osso C, Wagers AJ. Skeletal muscle stem cells: effects of aging and metabolism on muscle regenerative function. *Cold Spring Harb Symp Quant Biol* 2011, 76: 101–111.
16. Alway SE, Myers MJ, Mohamed JS. Regulation of satellite cell function in sarcopenia. *Front Aging Neurosci* 2014, 6: 246.
17. Price FD, von Maltzahn J, Bentzinger CF, Dumont NA, Yin H, Chang NC, Wilson DH, *et al*. Inhibition of JAK-STAT signaling stimulates adult satellite cell function. *Nat Med* 2014, 20: 1174–1181.
18. Cosgrove BD, Gilbert PM, Porpiglia E, Mourkioti F, Lee SP, Corbel SY, Llewellyn ME, *et al*. Rejuvenation of the muscle stem cell population restores strength to injured aged muscles. *Nat Med* 2014, 20: 255–264.
19. Gao CQ, Zhi R, Yang Z, Li HC, Yan HC, Wang XQ. Low dose of IGF-I increases cell size of skeletal muscle satellite cells via Akt/S6K signaling pathway. *J Cell Biochem* 2015, 116: 2637–2648.
20. Zhang L, Wang XH, Wang H, Du J, Mitch WE. Satellite cell dysfunction and impaired IGF-1 signaling cause CKD-induced muscle atrophy. *J Am Soc Nephrol* 2010, 21: 419–427.
21. Chakravarthy MV, Davis BS, Booth FW. IGF-I restores satellite cell proliferative potential in immobilized old skeletal muscle. *J Appl Physiol* 2000, 89: 1365–1379.
22. Eisenberg I, Eran A, Nishino I, Moggio M, Lamperti C, Amato AA, Lidov HG, *et al*. Distinctive patterns of microRNA expression in primary muscular disorders. *Proc Natl Acad Sci USA* 2007, 104: 17016–17021.
23. Greco S, De Simone M, Colussi C, Zaccagnini G, Fasanaro P, Pescatori M, Cardani R, *et al*. Common micro-RNA signature in skeletal muscle damage and regeneration induced by Duchenne muscular dystrophy and acute ischemia. *FASEB J* 2009, 23: 3335–3346.
24. McCarthy JJ. The MyomiR network in skeletal muscle plasticity. *Exerc Sport Sci Rev* 2011, 39: 150–154.
25. Briata P, Lin WJ, Giovarelli M, Pasero M, Chou CF, Trabucchi M, Rosenfeld MG, *et al*. PI3K/AKT signaling determines a dynamic switch between distinct KSRP functions favoring skeletal myogenesis. *Cell Death Differ* 2012, 19: 478–487.
26. Wu R, Li H, Zhai L, Zou X, Meng J, Zhong R, Li C, *et al*. MicroRNA-431 accelerates muscle regeneration and ameliorates muscular dystrophy by targeting Pax7 in mice. *Nat Commun* 2015, 6: 7713.
27. Eichner LJ, Perry MC, Dufour CR, Bertos N, Park M, St-Pierre J, Giguere V. miR-378(*) mediates metabolic shift in breast cancer cells via the PGC-1beta/ERRgamma transcriptional pathway. *Cell Metab* 2010, 12: 352–361.
28. Carrer M, Liu N, Grueter CE, Williams AH, Frisard MI, Hulver MW, Bassel-Duby R, *et al*. Control of mitochondrial metabolism and systemic energy homeostasis by microRNAs 378 and 378*. *Proc Natl Acad Sci USA* 2012, 109: 15330–15335.
29. Zhang Y, Li C, Li H, Song Y, Zhao Y, Zhai L, Wang H, *et al*. miR-378 activates the pyruvate-PEP futile cycle and enhances lipolysis to ameliorate obesity in mice. *EBioMedicine* 2016, 5: 93–104.
30. Mandillo S, Tucci V, Holter SM, Meziane H, Banchaabouchi MA, Kallnik M, Lad HV, *et al*. Reliability, robustness, and reproducibility in mouse behavioral phenotyping: a cross-laboratory study. *Physiol Genomics* 2008, 34: 243–255.
31. Capers PL, Hyacinth HI, Cue S, Chappa P, Vikulina T, Roser-Page S, Weitzmann MN, *et al*. Body composition and grip strength are improved in transgenic sickle mice fed a high-protein diet. *J Nutr Sci* 2015, 4: e6.
32. Sacheck JM, Ohtsuka A, McLary SC, Goldberg AL. IGF-I stimulates muscle growth by suppressing protein breakdown and expression of atrophy-related ubiquitin ligases, atrogin-1 and MuRF1. *Am J Physiol Endocrinol Metab* 2004, 287: E591–E601.
33. Wieteska-Skrzeczynska W, Grzelkowska-Kowalczyk K, Tokarska J, Grabiec K. Growth factor and cytokine interactions in myogenesis. Part I. The effect of TNF-alpha and IFN-gamma on IGF-I-dependent differentiation in mouse C2C12 myogenic cells. *Pol J Vet Sci* 2011, 14: 417–424.
34. Shima A, Pham J, Blanco E, Barton ER, Sweeney HL, Matsuda R. IGF-I and vitamin C promote myogenic differentiation of mouse and human skeletal muscle cells at low temperatures. *Exp Cell Res* 2011, 317: 356–366.
35. De Arcangelis V, Coletti D, Conti M, Lagarde M, Molinaro M, Adamo S, Nemoz G, *et al*. IGF-I-induced differentiation of L6 myogenic cells requires the activity of cAMP-phosphodiesterase. *Mol Biol Cell* 2003, 14: 1392–1404.
36. Gagan J, Dey BK, Layer R, Yan Z, Dutta A. MicroRNA-378 targets the myogenic repressor MyoR during myoblast differentiation. *J Biol Chem* 2011, 286: 19431–19438.
37. Ju H, Yang Y, Sheng A, Qi Y. MicroRNA-378 promotes myogenic differentiation by targeting BMP4. *Mol Med Rep* 2016, 13: 2194–2200.
38. Liu HH, Wang JW, Zhang RP, Chen X, Yu HY, Jin HB, Li L, *et al*. In ovo feeding of IGF-1 to ducks influences neonatal skeletal muscle hypertrophy and muscle mass growth upon satellite cell activation. *J Cell Physiol* 2012, 227: 1465–1475.
39. Gomes MD, Lecker SH, Jagoe RT, Navon A, Goldberg AL. Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. *Proc Natl Acad Sci USA* 2001, 98: 14440–14445.