MicroRNA-206 is overexpressed in the diaphragm but not the hindlimb muscle of mdx mouse

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MicroRNAs are highly conserved, noncoding RNAs involved in posttranscriptional gene silencing. MicroRNAs have been shown to be involved in a range of biological processes, including myogenesis and muscle regeneration. The objective of this study was to test the hypothesis that microRNA expression is altered in dystrophic muscle, with the greatest change occurring, of the muscles examined, in the diaphragm. The expression of the muscle-enriched microRNAs was determined in the soleus, plantaris, and diaphragm muscles of control and dystrophin-deficient (mdx) mice by semiquantitative PCR. In the soleus and plantaris, expression of the mature microRNA 133a (miR-133a) and miR-206, respectively, was decreased by ~25%, whereas in the diaphragm, miR-206 expression increased by 4.5-fold relative to control. The increased expression of miR-206 in the mdx diaphragm was paralleled by a 4.4-fold increase in primary miRNA-206 (pri-miRNA-206) transcript level. Expression of Myod1 was elevated 2.7-fold only in the mdx diaphragm, consistent with an earlier finding demonstrating Myod1 can activate pri-miRNA-206 transcription. Transcript levels of Drosha and Dicer, major components of microRNA biogenesis pathway, were unchanged in mdx muscle, suggesting the pathway is not altered under dystrophic conditions. Previous in vitro analysis found miR-206 was capable of repressing utrophin expression; however, under dystrophic conditions, both utrophin transcript and protein levels were significantly increased by 69% and 3.9-fold, respectively, a finding inconsistent with microRNA regulation. These results are the first to report alterations in expression of muscle-enriched microRNAs in skeletal muscle of the mdx mouse, suggesting microRNAs may have a role in the pathophysiology of muscular dystrophy.

Muscular dystrophy; posttranscriptional; gene regulation

The muscular dystrophies are a broad class of inherited myopathies distinguished by debilitating muscle wasting that often results in death as a result of respiratory complications (4). The most prevalent dystrophy is Duchenne muscular dystrophy (DMD), an X-linked recessive disease caused by mutations in the dystrophin gene. Dystrophin is a large cos- tumeric protein that links the cytoskeleton and the basal lamina of the extracellular matrix (7). Loss of dystrophin protein in DMD leads to membrane destabilization and subsequent activation of pathophysiological processes, resulting in inflammation, necrosis, and fibrosis (13). Although the genetic defect responsible for DMD is known, the development of an effective treatment continues to be hampered by an incomplete understanding of the pathogenesis of the disease.

Microarray analyses of muscle from the dystrophin-deficient (mdx) mouse, an animal model of DMD, suggest that changes in gene expression may contribute to the pathophysiology of muscular dystrophy (17, 30, 31, 37, 40). However, surprisingly little is known about the mechanisms, both transcriptional and translational, that lead to altered gene expression under dystrophic conditions. In the last few years, a class of highly conserved small, noncoding RNAs, known as microRNAs (miRNAs), has been identified and shown to posttranscriptionally repress gene expression (9, 18). miRNAs silence gene expression by binding to the 3′-UTR of target miRNAs and either inhibit initiation of translation or promote cleavage of the transcript (18). A recent pattern-based analysis of worm, fly, mouse, and human genome found between 74 and 92% of the transcriptome is potentially regulated by miRNAs, suggesting miRNAs have an important role in the regulation of gene expression (25).

A number of muscle-enriched miRNAs have been identified and shown to be involved in cardiogenesis, myogenic differentiation, and growth (10, 19, 21, 24, 26, 41, 46). Chromatin immunoprecipitation experiments have provided evidence that is consistent with the idea that expression of muscle-enriched miRNAs is controlled by the myogenic regulatory factors Myod1 and myogenin (34). More recently, Rosenberg et al. (36) demonstrated that miRNA-206 is indeed a direct transcriptional target of Myod1. Given the emerging importance of miRNAs in regulating gene expression in striated muscle, the goal of the present study was to determine whether expression of the muscle-enriched miRNAs was altered in skeletal muscles of the mdx mouse. As a corollary, it was hypothesized that miRNA expression would be altered the greatest in the mdx diaphragm, given that it is the most severely affected muscle in the dystrophin-deficient mouse. In support of our hypothesis, the most significant finding of the study was the dramatic increase in miRNA-206 expression in the mdx diaphragm that was associated with a similar increase in Myod1 expression. Collectively, the results suggest the intriguing possibility that increased miR-206 expression contributes to the chronic pathology observed in the mdx diaphragm by repressing expression of genes that otherwise would serve a compensatory function, limiting the severity of the disease, as in the hindlimb musculature.

METHODS

Animal care. The use of animals was approved by the University of Kentucky Institutional Animal Care and Use Committee. Eight-week-old male control (strain C57BL/10ScSnJ) and mdx (strain C57BL/
10ScSn-Dmd-mdx(J) mice were purchased from Jackson Laboratory (Bar Harbor, ME). An age of 8 wk was chosen based on a microarray study that found the change in gene expression in the mdx hindlimb and diaphragm muscles peaked at this age (32). On arrival, mice were

**Tissue collection and RNA isolation.** Mice were killed by CO₂ asphyxiation, and soleus, plantaris, and diaphragm muscles were quickly dissected, frozen in liquid nitrogen, and stored at −80°C. Total RNA was isolated from muscle samples using TRIzol (Invitrogen, Carlsbad, CA), according to manufacturer’s directions. To remove genomic DNA contamination, all RNA samples were treated with TURBO DNase (Ambion, Austin, TX).

**Detection of mature miRNAs.** To detect mature miRNAs (miRs) in total RNA samples, the mirVana qRT-PCR miRNA detection kit and mirVana PCR primer sets for miR-1, -24, -133a, and -206 were used according to the manufacturer’s directions (Ambion, Austin, TX). Briefly, reverse transcriptase (RT) reactions were performed with miR-specific RT primers and 25 ng of total RNA for 30 min at 37°C, followed by 10-min incubation at 95°C to inactivate the RT enzyme. End-point PCR was then performed using the RT product and miR-specific primer for 20 cycles, except for detection of miR-206 in the plantaris, which required 25 cycles (two-step: 95°C for 15 s followed by 60°C for 30 s). To account for possible differences in the amount of starting RNA, all samples were normalized to the ubiquitously expressed miR-24.

**RT-PCR analysis.** Semiquantitative PCR was used to detect primary miRNA-206 (pri-miRNA-206), Myod1, Drosha, Exportin-5, Dicer1 and Utophin A transcript levels. First-strand cDNA synthesis from total RNA was performed with oligo(dT)12–18 primer using SuperScript II RT (Invitrogen, Carlsbad, CA), according to manufacturer’s directions. One microliter of the RT reaction was used for end-point PCR analysis, with PCR primers designed using Blaster Workbench 3.2 PrimerTm program (http://seqtool.sdsc.edu/CGI/BW.cgi). All primers were designed with melting temperature of ~60°C that amplified ~200 bp of the 3’ end of the coding sequence, except utrophin A primers, which were described by Angus et al. (2). Primers specific for pri-miRNA-206 were designed to target sequences 170 bp 5’ and 3’ to the miRNA stem-loop (pre-miRNA), as described by Cai et al. (6). After an initial denaturing step at 94°C for 3 min, the following amplification conditions were used: denature, 94°C for 30 s; anneal, 58°C for 30 s; extend, 72°C for 30 s. Thirty cycles were performed for each gene except utrophin, which required 40 cycles, and ribosomal protein L26 (Rpl26), which required 25 cycles. Serial dilution of the cDNA ensured these amplification conditions were in the linear range (data not shown). Primer sequences for each gene were as follows: Drosha, forward 5’-GGATAGCCGTGGAGGAAAGGA-3’, reverse 5’-CTCCTTGTATGCTCAGCTCCTC-3’; exportin-5, forward 5’-CCACCTCAACGCCTAATCGCT-3’; reverse 5’-GCCGAGGAGGATGCC-3’; Dicer1, forward 5’-TGGCTCGAGATG-3’; reverse 5’-TACGCTTGAACGTTAGG-3’; Myod1, forward 5’-GGAGCATAGTTGACCTGAAAC-3’; reverse 5’-GCCG-GAGAAGAGGATGCC-3’; utrophin A, forward 5’-GCCCGCGCTCAGCTGCTC3’; reverse 5’-CC- TACGCTTGAACGTTAGG-3’; utrophin A, forward 5’-CGGAGGAGGATGCCAAGGT-3’; reverse 5’-CTGCTAGCAGCAATCG-3’. To account for any difference in the amount of starting RNA, Rpl26 (forward 5’-CGAGTCCAGGAGGAGA-3’; reverse 5’-GACGTCTTTAATGAAAGCCTG-3’) was chosen as our endogenous control to normalize gene expression.

**PCR product quantification.** PAGE was used to quantify PCR products. The PCR reaction (7.5 µl of 30-µl volume) was loaded onto a 5% gel and run for 1 h at 40 V at room temperature with 0.5× Tris-borate-EDTA running buffer. The gel was then stained for 30 min at room temperature in SYBRgreen I (Invitrogen, Carlsbad, CA), according to the manufacturer’s directions. After staining, gels were imaged using Storm 860 (GE Healthcare, Piscataway, NJ), and bands representing PCR product were quantified using ImageQuant.

**Protein assay and Western blot analysis.** Whole muscle homogenates were prepared from control and mdx diaphragm muscles by polytron (PowerGen 125; Fischer Scientific, Suwanee, GA) in homogenization buffer [1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM NaCl, 400 mM KCl, 25 mM β-glycerophosphate, 50 mM NaF, 5 mM benzamidine, 20 mM Tris-HCl (pH 7.6), 1 mM EDTA, 1 mM sodium orthovanadate, 5 mM N-ethylmaleimide, 1 mM phenylmethylsulphonyl fluoride] supplemented with protease inhibitor cocktail (Sigma, St. Louis, MO; catalog no. P8340). The muscle homogenates were then centrifuged for 10 min at 10,000 g at 4°C, and the protein concentration of the supernatant was determined using the Bradford protein assay (Bio-Rad Laboratories, Hercules, CA). Fifty micrograms of whole muscle homogenate per sample were separated by SDS-PAGE (5% gel) and then transferred to nitrocellulose membrane (0.2 µm) (Bio-Rad, Hercules, CA). The membrane was incubated in blocking buffer [5% nonfat dry milk in Tris-buffered saline (TBS) plus 0.1% tween-20 (TBS-T)] for 1 h at room temperature and then incubated in blocking buffer overnight at 4°C with a 1:500 dilution of an utrophin antibody. The utrophin antibody (MANCHO3 clone 8A4) was developed by Dr. G. E. Morris (27) and obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the National Institute of Child Health and Human Development and maintained by The University of Iowa, Department of Biological Sciences, Iowa City, IA. After the overnight incubation, the membrane was washed for 5 min four times in TBS-T and then incubated with anti-mouse horseradish peroxidase-conjugated secondary antibody (2 ng/ml) for 45 min at room temperature in blocking buffer. Following this incubation, the membrane was washed again in TBS-T, as described above, and then incubated for 4 min in SuperSignal West Femto Maximum Sensitivity Substrate (Pierce, Rockford, IL) and exposed to X-ray film. Analysis of band intensity was performed using ImageJ software (http://rsb.info.nih.gov/ij/index.html) on the scanned film. A separate gel run under identical conditions as described above was stained with Coomassie brilliant blue R-250 to confirm equal protein loading, as judged by myosin heavy chain content.

**Statistical analysis.** Data are reported as means ± SE with n = 4–5 muscles. Student’s t-test was used to determine whether a significant difference existed between control and mdx samples for each of the variables examined. A significant difference (P < 0.05) was denoted by an asterisk.

**RESULTS**

miR-206 expression is increased in mdx diaphragm. The expression levels of three muscle-enriched miRs were determined in hindlimb muscles (soleus and plantaris) and diaphragm of control and mdx mice. As shown in Fig. 1, A and B, there were no differences in the expression of miR-1 or miR-133a in any of the muscles examined, except in the mdx soleus muscle, where miR-133a expression was modestly decreased by 23%. In striking contrast, miR-206 expression was dramatically increased in the mdx diaphragm by 4.5-fold compared with control diaphragm (Fig. 1C). There was no difference observed in miR-206 expression between control and mdx soleus muscles, whereas, in the mdx plantaris, miR-206 expression was decreased by 29% compared with control (Fig. 1C).

pri-miRNA-206 expression is increased in the mdx diaphragm. The mature miR is derived from a pri-miRNA transcript through a series of endonuclease reactions, and, as a consequence, there is typically an 1:1 relationship between the abundance of pri-miRNA transcript and the mature miR, al-
though exceptions have been reported (6, 22). To determine whether this relationship remained true under dystrophic conditions pri-miRNA-206 expression was determined in the diaphragm of the mdx mouse. In complete agreement with dystrophic conditions pri-miRNA-206 expression was elevated 4.4-fold in the mdx diaphragm compared with control (Fig. 2). The minor loss in miR-206 expression in the mdx plantaris, however, was not paralleled by a similar decrease in pri-miRNA-206 expression, as no difference was detected between control and mdx plantaris muscles in pri-miRNA-206 transcript levels (Fig. 2).

*Drosa* and Dicer expression is not altered in the mdx diaphragm. miRNA expression can also be altered by a change in the miRNA biogenesis pathway (16, 44). Three proteins have been identified as having a major role in miRNA biogenesis: the two RNase III endonucleases Drosha and Dicer and the transport protein Exportin-5. Semiquantitative PCR analysis determined Drosha and Dicer transcript levels were not changed in the mdx diaphragm; in contrast, Exportin-5 transcript levels were increased by 31% (Fig. 3).

*Myod1* expression is increased in the mdx diaphragm. The transcription factor Myod1 has been shown to induce the expression of pri-miRNA-206 (36). Consistent with this finding, Myod1 expression was increased 2.7-fold in mdx diaphragm compared with control (Fig. 4). Furthermore, in the mdx plantaris, in which pri-miRNA-206 expression was unchanged, Myod1 expression was not different from control level (Fig. 4). Although correlative, these findings suggest that Myod1 is responsible for the increased expression of miR-206 in the mdx diaphragm by upregulating pri-miRNA-206 expression.

*Utrophin* expression is posttranscriptionally increased in the mdx diaphragm. A predicted target gene of miR-206 is utrophin (http://microrna.sanger.ac.uk/). Rosenberg et al. (36) confirmed this prediction with multiple lines of evidence showing miR-206 was capable of posttranscriptionally repressing utrophin expression. To determine whether miR-206 might function in a similar fashion under dystrophic conditions, we measured utrophin transcript and protein levels in mdx diaphragm. As shown in Fig. 5, A and B, utrophin transcript level increased by 69%, whereas utrophin protein abundance increased by 3.9-fold in the mdx diaphragm compared with control diaphragm. These results indicate utrophin is posttranscriptionally regulated in the mdx diaphragm; however, the results are not consistent with regulation by miR-206 as utrophin protein increased, not decreased as would be expected if regulated by miR-206.

**DISCUSSION**

The most significant finding of the study was the 4.5-fold increase in miR-206 expression in the mdx diaphragm. Somewhat surprisingly, the only other alterations in miR expression detected were modest decreases of 23 and 29% in miR-133a in the soleus and miR-206 in the plantaris, respectively (Fig. 1). Given that the diaphragm is the most affected muscle in the mdx mouse at this age suggested the elevated level of miR-206 may be contributing to the severity of disease by repressing the expression of genes whose function may otherwise serve to ameliorate the pathology (5, 12, 23). This proposition is supported by the finding that miR expression was only modestly changed in mdx hindlimb muscles, which do not display as severe a phenotype as the mdx diaphragm (38).

The primary objective of the present study was to determine whether expression of the muscle-enriched miRNAs miR-1,
miRNAs with altered expression during muscular dystrophy. The kinetics of satellite cell differentiation also appeared to be altered in mdx muscle. Yublonka-Revuevini and Anderson (45) found satellite cells derived from mdx diaphragm transitioned from a proliferative state to differentiated state more quickly compared with primary cultures derived from control diaphragm. The increased expression of miR-206 may contribute to the accelerated differentiation of mdx satellite cells. This idea is supported by a recent study that found increased expression of miR-206 promoted differentiation of C2C12 myoblasts by repressing the expression of the p180 subunit of DNA polymerase α (Pola1) (19). A proteomics analysis, however, did not identify Pola1 protein as differentially expressed in a comparison between control and mdx diaphragm, although the protein may be below the threshold of detection (11).

Fig. 2. Expression of primary miRNA-206 (pri-miRNA-206) in control and mdx muscles. pri-miRNA-206 expression was determined by semiquantitative PCR in control and mdx plantaris and diaphragm muscles. A: representative image of PCR product from control (C; lanes 1 and 3) and mdx (M; lanes 2 and 4) plantaris and diaphragm muscles. Expression of pri-miRNA-206 was normalized to ribosomal protein L26 (Rpl26). B: histogram of densitometric quantification of PCR product showing no change in expression in the mdx plantaris but a 4.4-fold (P = 0.0008) increase in the mdx diaphragm compared with control. Values are means ± SE (n = 4–5) expressed as fold difference relative to control muscle expression level. *Significant difference (P < 0.05).

-133a, and -206 were altered with dystrophin deficiency. The emerging importance of miRNAs in muscle differentiation, repair and growth, and their role in the development of other diseases such as cancer, provided a reasonable expectation that miRNA expression may be changed with muscular dystrophy (10, 19, 20, 26, 41). The following conclusions can be drawn from the results of this study: 1) the increased expression of miR-206 in the mdx diaphragm most likely reflects activation of pri-miRNA-206 transcription by Myod1; 2) expression of major components of the miRNA biogenesis pathway does not appear to be significantly altered with muscular dystrophy; and 3) under dystrophic conditions, the pattern of utrophin protein expression is not consistent with regulation by miR-206, contrary to such findings in vitro (36). These results are the first evidence of miRNAs’ potential role in the pathophysiology underlying muscular dystrophy and provide the basis for a more comprehensive study identifying the complete suite of miRNAs with altered expression during muscular dystrophy.

The mechanisms responsible for the increase of miR-206 expression in the mdx diaphragm remain unclear, but our results suggest that Myod1 may participate in this response. A number of studies have clearly established that pri-miRNA-206 is a direct transcriptional target of Myod1 (34, 36). Consistent with this finding, we observed a direct correlation between pri-miRNA-206 and Myod1 expression levels; compared with control diaphragm, pri-miRNA-206 and Myod1 expression were increased by 4.4-fold and 2.7-fold in the mdx diaphragm, respectively. In contrast, their expression levels were unchanged in the mdx plantaris (Fig. 2).

The upregulation of Myod1 transcription most likely reflects satellite cell activation associated with the cycle of muscle regeneration known to occur in the mdx diaphragm. This possible mechanism is supported by immunocytochemistry, which showed Myod1 expression was increased by approximately twofold in mdx muscle and was localized to regions of regeneration, presumably reflecting satellite cell activation (1). The expression of miRNAs’ potential role in the pathophysiology and their role in the development of other diseases such as cancer, provided a reasonable expectation that miRNA expression may be changed with muscular dystrophy. The following conclusions can be drawn from the results of this study: 1) the increased expression of miR-206 in the mdx diaphragm most likely reflects activation of pri-miRNA-206 transcription by Myod1; 2) expression of major components of the miRNA biogenesis pathway does not appear to be significantly altered with muscular dystrophy; and 3) under dystrophic conditions, the pattern of utrophin protein expression is not consistent with regulation by miR-206, contrary to such findings in vitro (36). These results are the first evidence of miRNAs’ potential role in the pathophysiology underlying muscular dystrophy and provide the basis for a more comprehensive study identifying the complete suite of miRNAs with altered expression during muscular dystrophy.

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Fig. 3. Transcript levels of components of the miRNA biogenesis pathway in control and mdx diaphragm. A: representative image of PCR product for Drosha, Exportin-5, and Dicer from control (C) and mdx (M) diaphragm. Expression of each transcript was normalized to Rpl26 expression. B: histogram of densitometric quantification of PCR product showing no change in Drosha or Dicer expression, whereas exportin-5 expression was increased by 31% (P = 0.003) in the mdx diaphragm relative to control. Values are means ± SE (n = 4–5) expressed as fold difference relative to control muscle expression level. *Significant difference (P < 0.05).
An alternative mechanism that could account for the increase in miR-206 is the structural denervation known to occur in the mdx diaphragm during muscle regeneration (28). Upon muscle regeneration, the alteration in the structure of the neuromuscular junction (i.e., reduced acetylcholine receptor clustering) is such that there is increased variability in synaptic transmission (8, 29). Support for this mechanism comes from a study in which a muscle-specific noncoding transcript (7H4) was cloned from a synaptically enriched cDNA library derived from the rat diaphragm (42). Surprisingly, miR-206 is now known to be derived from the 7H4 transcript in both the mouse and rat, and, upon denervation, 7H4 transcript abundance was increased (35, 42). Consequently, it may be the functional denervation that accompanies the cycle of muscle regeneration is responsible for the increase in miR-206 in the mdx diaphragm, independent of Myod1 activation.

What is the biological implication of increased miR-206 expression in the mdx diaphragm? Persistent miR-206 expression may contribute to the severe pathology observed in the mdx diaphragm by repressing the expression of genes that otherwise would normally function in a compensatory manner, as may happen in limb musculature. A predicted target of miR-206 that would support such a scenario is utrophin. The expression of utrophin is subject to a 3'-UTR-dependent post-transcriptional mechanism perfectly amenable to miRNA regulation (15, 43). Furthermore, miR-206 was recently shown to be capable of regulating utrophin expression in vitro (36). To determine whether indeed utrophin was under miR-206 regulation, we measured utrophin transcript and protein levels in control and mdx diaphragm. In agreement with earlier reports, we found evidence that suggested utrophin was posttranscriptionally regulated; utrophin transcript abundance increased 69%, whereas utrophin protein abundance increased 3.7-fold relative to control, respectively (Fig. 5). The relationship between utrophin transcript and protein levels, however, was opposite of what would be expected if utrophin expression were regulated by miR-206. What could account for this discrepancy? One possible explanation is the effect that the different experimental conditions under which utrophin expression was assessed. The dystrophic cellular environment is characterized by a metabolic crisis that leads to a heightened susceptibility to oxidative stress (31, 33). The cellular stress associated with muscular dystrophy may inhibit miRNA repression of translation, as has been shown in human hepatocarcinoma cells subjected to starvation (amino acid depletion) or oxidative stress (exposure to 0.1 mM sodium arsenite) (3).

It may be that, under dystrophic conditions, miR-206 is not able to effectively repress translation of utrophin mRNA.

Fig. 4. Myod1 expression in control and mdx muscles. Myod1 transcript level was determined by semiquantitative PCR in control and mdx plantaris and diaphragm muscles. A: representative image of PCR product from control (C; lanes 1 and 3) and mdx (M; lanes 2 and 4) plantaris and diaphragm muscles. Expression of Myod1 was normalized to Rpl26. B: histogram of densitometric quantification of Myod1 PCR product showed no change in expression in the mdx plantaris but a 2.7-fold (P = 0.001) increase in the mdx diaphragm compared with control. Values are means ± SE (n = 4–5) expressed as fold difference relative to control muscle expression level. *Significant difference (P < 0.05).

Fig. 5. Utrophin mRNA and protein expression in control and mdx diaphragm. A: representative image of semiquantitative PCR product from control (C, lane 1) and mdx (M, lane 2) diaphragm. Utrophin mRNA expression was normalized to Rpl26. B: representative Western blot image from control (C, lane 1) and mdx (M, lane 2) diaphragm. Utrophin protein expression was normalized to myosin heavy chain content. C: histogram of densitometric quantification of utrophin PCR product and utrophin protein revealed a 69% increase mRNA expression (P = 0.043) with a 3.9-fold (P = 0.018) increase in utrophin protein in the mdx diaphragm compared with control. Values are means ± SE (n = 4–5) expressed as fold difference relative to control muscle expression level. *Significant difference (P < 0.05).
To identify other potential targets of miR-206 that might normally serve some compensatory function, we screened genes that have been previously shown by microarray or proteomic analysis to be downregulated with muscular dystrophy (11, 31, 37, 39). Of the roughly 50 genes screened, only two were found to be predicted targets of miR-206: fatty acid synthase (Fasn) and stearoyl CoA desaturase (Scd1). A search of the KEGG database (release 41.1; www.genome.jp/kegg/) revealed these two genes are part of the lipogenic pathways and, as suggested by Tkatchenko et al. (39), may contribute to altered energy metabolism observed in mdx muscle (14). It seems unlikely, however, that miR-206 is involved in regulating dystrophy.

The primary aim of this study was to test the hypothesis that expression of the muscle-enriched miRNAs would be altered in skeletal muscle of the dystrophin-deficient mouse. The results confirmed our hypothesis as the muscle-enriched miRNA miR-206 was significantly elevated in the diaphragm of the mdx mouse. The fact that miR-206 expression was only increased in the mdx diaphragm, and not the hindlimbs muscles, offers the intriguing possibility that miR-206 may have a role in the chronic pathology known to affect the diaphragm of the mdx mouse. The results of this study provide the impetus for identifying the complete set of miRNAs whose expression is changed with muscular dystrophy. The identification of such a miRNA “signature” for muscular dystrophy is expected to provide a list of common target genes whose misregulation by miRNAs may contribute to the pathophysiology of muscular dystrophy.

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