The role of microRNAs in skeletal muscle health and disease

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1. ABSTRACT

Over the last decade non-coding RNAs have emerged as importance regulators of gene expression. In particular, microRNAs are a class of small RNAs of \textasciitilde22 nucleotides that repress gene expression through a post-transcriptional mechanism. MicroRNAs have been shown to
be involved in a broader range of biological processes, both physiological and pathological, including myogenesis, adaptation to exercise and various myopathies. The purpose of this review is to provide a comprehensive summary of what is currently known about the role of microRNAs in skeletal muscle health and disease.

2. INTRODUCTION

2.1. History

Initially described as a small RNA involved in developmental timing, the study of the first microRNA (miRNA) lin-4 was carried out in relative obscurity in the early 1990s, of real interest to only those in the nematode (Caenorhabditis elegans) community (1,2). These pioneering studies by the Ruvkun and Ambros laboratories provided the first evidence demonstrating that lin-14 expression was regulated by a post-transcriptional mechanism involving the interaction between the lin-14 3′-UTR and the small non-coding RNA lin-4 (3,4). Pasquinelli and colleagues reported in late 2000 the identification of let-7, a second "small temporal RNA" (stRNA) from C. elegans that down-regulated lin-41 expression, and unlike lin-4, was detected in a broad range of bilaterian animals including vertebrate, ascidian, hemichordate, mollusc, annelid and arthropod (5). The discovery that let-7 was phylogenetically conserved in bilaterian animals was a major milestone in the history of the miRNA field as it strongly suggested the post-transcriptional regulation of gene expression by small RNAs was more widespread than just C. elegans. Shortly thereafter, three independent reports described the identification of 30-50 new miRNAs in the human, fly and worm, providing additional support for the idea that miRNAs may have an important role in the regulation of gene expression in animals (6-9). The prescient nature of these early findings is revealed in the latest release of miRBase (version 20, June, 2013; www.mirbase.org) which catalogs 30,424 miRNA sequences from 206 species with 2578 and 1908 human and mouse miRNAs, respectively.

2.2. Biogenesis

The vast majority of miRNAs are the product of RNA polymerase II transcription resulting in a primary miRNA (pri-miRNA) transcript that has the characteristic 5′m7G cap structure and 3′ poly(A) tail (10,11). Recent genomic mapping confirmed an earlier study showing that roughly half of annotated miRNAs are intragenic (exon, intron, 3′-UTR or 5′-UTR), located within protein-coding or non-coding RNA (ncRNA) genes (12,13). In general, miRNA expression parallels the host gene, though new experimental evidence indicates that up 30% of intronic miRNAs are expressed as an independent transcription unit under regulation of its own promoter (14). Once transcribed, the pri-miRNA forms a stem-loop structure that is recognized by the microprocessor complex which contains two core components, the RNase III endonuclease Drosha and the double-stranded RNA binding protein DGCR8 (Di George Syndrome critical region gene 8) (15, 16). DGCR8 binds to the stem-loop structure and then guides Drosha into position, cleaving ~11 base pairs (bp) from the base of the stem-loop to produce a 60-70 bp hairpin RNA molecule designated the precursor miRNA (pre-miRNA) (17). The generation of certain pre-miRNAs have been shown to be regulated by proteins that are not core components of the microprocessor complex (18). For example, SMAD interacts with p68, a RNA helicase associated with the microprocessor complex, to promote production of miR-21 which is known to regulate AKT activity by targeting PTEN (phosphatase and tensin homolog) (19, 20). Given the importance of TGF-β (transforming growth factor-β) signaling in the regulation of skeletal muscle mass, it would be of interest to see if such a similar mechanism is operative during periods of muscle growth.

Following pri-miRNA processing, the 60-70 bp precursor miRNA (pre-miRNA) is transported from the nucleus by Exportin 5, a nuclear export receptor, to the cytoplasm (21, 22). Once in the cytoplasm, a second RNase III endonuclease, Dicer, cleaves the pre-miRNA to produce ~22
nucleotide double-stranded RNA molecule in which one strand, known as the guide strand, is transferred to RISC (RNA-induced silencing complex) containing Argonaute 2 (Ago2) and the RNA binding protein Tarbp2 (TAR (HIV) RNA binding protein 2); the other strand is typically targeted for degradation (23). The mature miRNA directs RISC to 3′-UTR of target mRNA through complementary binding of the miRNA seed sequence which results in inhibition of translation and/or degradation of the target transcript (24). As with Drosha, Dicer activity has been shown to be modified by its association with different factors (25). In particular, the cold-shock protein RBM3 (RNA binding motif protein 3) has been shown to promote pre-miRNA association with Dicer leading to an increase in mature miRNA expression; interestingly, Dupont-Versteegden and coworkers reported RBM3 appears to be capable of regulating muscle size (26, 27).

Although the vast majority of miRNAs are generated through the aforementioned pathway, there are examples of miRNAs being produced independent of Drosha and Dicer (28, 29). Cheloufi and colleagues made the unexpected discovery that miR-451 could be generated through the processing of pre-miRNA-451 by Ago2 (30). Interestingly, expression of miR-451 has been reported to decrease with age in skeletal muscle and be increased during differentiation of human myoblasts as well as in low responders to resistance exercise (31, 32). What remains to be reconciled with these intriguing findings is the observation, using a mouse β-galactosidase reporter strain, that miR-451 expression is restricted to the circulatory system, consistent with miR-451 known role in erythropoiesis (33). Though speculative, it may be that some of the findings reported for miR-451 in skeletal muscle reflect concomitant changes in the vascular system and not the muscle itself per se.

### 2.3. Tissue-specific expression

Almost immediately upon discovering that miRNAs were conserved across species, came the realization that some miRNAs were not ubiquitously expressed as *let-7*, but were expressed only in certain tissues. One of the first examples of a tissue-specific miRNA was miR-1 which was found to be expressed exclusively in the human heart but not in the brain, kidney, liver, lung or HeLa cells (6, 8). The initial finding that some miRNAs were expressed in a tissue-specific fashion was confirmed in a study by Lagos-Quintana and coworkers (2002) showing that miR-1, -122a and -124a expression was restricted to striated muscle, liver and brain, respectively (9). In an effort to identify new miRNAs, Sempere et al. (2004) identified 30 miRNAs that were enriched or specifically expressed within a particular tissue (34). These authors provided the first description of striated muscle-specific miR-1, -133a and -206, which were later designated as myomiRs (34, 35).

The myomiR family has expanded since its original description to include miR-208a, miR-208b, miR-499 and, most recently, miR-486 (36-38). Northern blot analyses showed that these new members of the myomiR family are strictly striated muscle-specific (miR-208a, miR-208b and miR-499), being derived from the intron of different muscle-specific myosin heavy chain genes, or highly enriched in muscle (miR-486) (36, 37). Most myomiR family members are expressed in both the heart and skeletal muscle except for miR-208a, which is cardiac-specific, and miR-206, which is skeletal muscle-specific and enriched in slow-twitch muscles such as the soleus (39).

### 2.4. MyomiRs

Since their original description, a great of effort has gone into determining the function of myomiRs in striated muscle (reviewed in Liu and Olson, 2010) (40). The finding that overexpression of miR-1 was capable of promoting a shift to a myogenic profile in HeLa cells indicated that myomiRs may have a fundamental role in muscle biology (41). The importance of myomiRs in the regulation of myogenesis was first demonstrated by Sokol and Ambros (2005) who reported the deletion of miR-1 in the fly resulted in premature death resulting from incomplete skeletal muscle growth during early development (42). Interestingly, myomiRs...
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appear to have either uniform expression throughout the muscle (miR-1 and miR-133a), independent of fiber-type, or are enriched in slow-twitch, type I muscles (miR-206, miR-208b and miR-499) (37, 39); to date, no myomiR has been reported to be enriched specifically in fast-twitch, type II muscle. However, RNA-seq analysis of porcine and bovine skeletal muscle has identified non-myomiRs that are relatively enriched in a muscle of a particular fiber type (43, 44). For instance, Muroya and colleagues (2013) reported that in steers miR-885 and miR-196a were highly enriched in the fast-twitch semitendinosus muscle relative to the slow-twitch masseter muscle (44).

Gene targeting studies have shown that deletion of individual myomiRs in the mouse has had surprisingly little impact on skeletal muscle phenotype (37, 38, 45-49). For example, deletion of skeletal muscle-specific miR-206 resulted in no obvious phenotype as reflected by soleus muscle weight, morphology or fiber-type distribution; however, recovery from denervation was delayed in muscle of the miR-206 knockout (47). In a similar manner, the Olson laboratory showed that miR-208a was necessary for the stress response involved in cardiac hypertrophy (38). Collectively, these findings from myomiR knockout mice are consistent with the notion that a primary function of miRNAs is to mediate the stress response of the cell by helping to restore homeostasis through regulating gene expression (50); however, why the myomiR knockout mice do not show a more dramatic phenotype as predicted from in vitro studies remain unclear (51, 52). One possible explanation could be the overlap in target genes among myomiR family members, such as miR-1 and miR-206, which in theory could rescue any deleterious phenotype resulting from the miRNA knockout. This idea is supported by the double knockout of miR-133a-1 and miR-133a-2 in which mice showed septal defects and skeletal muscle myopathy that was not present in the single miR-133a knockout mice (53, 54).

The purpose of this review is to, first, present the current knowledge about the role of miRNAs during skeletal muscle formation and regeneration, as well as in response to muscular activity. Secondly, the review will summarize the functions of miRNAs in skeletal muscle disorders, including dystrophies and myopathies, disorders related to disease and disuse, and sarcopenia.

3. MICRONRNA REGULATION OF MYOGENESIS

It is now well-established that miRNAs play an integral role in skeletal muscle development, particularly through their regulation of myogenic progenitor cells. Myogenesis is an extremely complex biological process that is beyond the scope of this review; for a comprehensive overview of myogenesis, the reader is kindly referred to these in-depth reviews (55-58). Briefly, during skeletal muscle development, two upstream regulatory factors, Pax3 (paired box 3) and Pax7 (paired box 7), are expressed in progenitor cells during the determination and formation of skeletal muscle (for recent reviews see 58). Furthermore, the fate of these cells is controlled by a complex network of basic helix–loop–helix muscle regulatory factors (MRFs) which include MYOD (myogenic differentiation), MYF5 (myogenic factor 5), myogenin, and MRF4 (myogenic regulatory factor 4) (for comprehensive reviews see 58-61), and it is primarily through the regulation of these transcription factors that miRNAs exert their biological effect in skeletal muscle. Additionally, Mef2 (Myocyte enhancer factor 2) transcription factors are also important during muscle differentiation (for review see 62), and have been shown to be regulated by miRNAs (63-66).

To date, most of the work that has contributed to our understanding of miRNAs during myogenesis has been performed using the myogenic C2C12 cell line. This cell line readily recapitulates the proliferation and differentiation processes that myogenic progenitor cells undergo during formation of skeletal muscle in vivo. However, following postnatal development, myogenic cells transition into a state of quiescence, which is difficult to
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study using an in vitro model. Due to these limitations, only recently have reports emerged implicating miRNAs during the transition and maintenance of myogenic cells into this state of quiescence (67, 68). During this post-natal period, these cells specifically express the transcription factor Pax7 and are referred to as satellite cells (61). Furthermore, while these cells normally remaining quiescent, upon injury, satellite cells become activated, enter the cell cycle, and proliferate, serving as the cellular basis for muscle repair. Therefore, miRNAs play a role not only in the development of skeletal muscle, but also during regeneration following muscle injury, a process that recapitulates the different stages of myogenesis. The miRNAs and their respective targets that have been experimentally-shown to regulate myogenesis are listed in Table 1. Moreover, a summary of our current understanding of the major miRNAs involved in the regulation of myogenesis is shown in Figure 1.

3.1. Proliferation

The early work by Chen and colleagues (2006) was one of the first to provide evidence indicating that miRNAs regulate the proliferative behavior of myogenic cells (52). These authors reported that over-expression of miR-133a promoted myoblast proliferation by repressing the expression of Srf (serum response factor) (52). Similarly, miR-27a has since been shown to be involved in promoting myoblast proliferation by targeting a well-known negative regulator of myogenesis, myostatin (69). The regulation of cell proliferation through myostatin appears to be through an auto-regulatory loop involving the myostatin downstream target Smad3/4 complex; activation of the Smad3/4 complex, in turn, leads to miR-27a induction which then feeds back to inhibit myostatin expression (70). Microarray analysis of proliferating myogenic cells revealed that miR-682 is up-regulated, with functional assays demonstrating that inhibition of miR-682 is
### Table 1. MicroRNAs controlling myogenic progenitor cell fate

<table>
<thead>
<tr>
<th>Cell State</th>
<th>miRNA</th>
<th>Targets</th>
<th>Biological Role of miRNA</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quiescence</td>
<td>miR-489</td>
<td>Dek</td>
<td>Regulation of the proliferation of daughter cells primed for differentiation following asymmetrical division</td>
<td>67</td>
</tr>
<tr>
<td>Quiescence</td>
<td>miR-31</td>
<td>Myf5</td>
<td>Suppression of Myf5 protein levels prior to signals for differentiation</td>
<td>68</td>
</tr>
<tr>
<td>Proliferation</td>
<td>miR-133a</td>
<td>Srf</td>
<td>Promotes proliferation by repressing SRF, a transcription factor involved in regulating the expression for differentiation</td>
<td>52</td>
</tr>
<tr>
<td>Proliferation</td>
<td>miR-27a</td>
<td>Myostatin</td>
<td>Promotes myogenesis by relieving the negative regulation of myostatin</td>
<td>69</td>
</tr>
<tr>
<td>Proliferation</td>
<td>miR-27b</td>
<td>Pax3, Mef2c</td>
<td>Promotes proper migration of myogenic progenitor cells by suppressing Mef2c levels thereby not allowing association with myogenic regulatory factors</td>
<td>98, 97</td>
</tr>
<tr>
<td>Differentiation</td>
<td>miR-1</td>
<td>Pax7</td>
<td>Promotes differentiation by downregulating Pax7 and the genes under its control</td>
<td>72-73</td>
</tr>
<tr>
<td>Differentiation</td>
<td>miR-133</td>
<td>Sp1, Fgfr1, Pp2ac</td>
<td>Promotes cell cycle arrest through downregulation of SP1 target, CyclinD1, inhibits proliferation through suppression of ERK1/2 signaling</td>
<td>75, 80</td>
</tr>
<tr>
<td>Differentiation</td>
<td>miR-206</td>
<td>Subunits of DNA Pola</td>
<td>Promotes differentiation by downregulating Notch signaling, which normally acts to inhibit premature differentiation</td>
<td>64</td>
</tr>
<tr>
<td>Differentiation</td>
<td>miR-486</td>
<td>Pax7</td>
<td>Promotes differentiation by downregulating Pax7 and the genes under its control</td>
<td>73</td>
</tr>
<tr>
<td>Differentiation</td>
<td>miR-26a</td>
<td>EzH2, Smad1/4</td>
<td>Promotes activation of mTOR signaling by relieving inhibitory effects of PTEN on the pathway</td>
<td>116</td>
</tr>
<tr>
<td>Differentiation</td>
<td>miR-214</td>
<td>EzH2</td>
<td>Relieves the repressive effects of Polycomb complex on myogenic genes</td>
<td>82</td>
</tr>
<tr>
<td>Differentiation</td>
<td>miR-214</td>
<td>EzH2</td>
<td>Inhibits TGF-β signaling to promote myogenesis</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N-Ras</td>
<td>Promotes cell cycle arrest</td>
<td>84</td>
</tr>
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Table 1. Contd...

<table>
<thead>
<tr>
<th>Cell State</th>
<th>miRNA</th>
<th>Targets</th>
<th>Biological Role of miRNA</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MiR-503</td>
<td>Cdc25A</td>
<td>Promotes cell cycle arrest</td>
<td>87</td>
</tr>
<tr>
<td>miR-29 b/c</td>
<td></td>
<td>Yy1</td>
<td>Relieves inhibitory effects of NFκB on myogenesis</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rybp</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hdac4</td>
<td>Relieves the repressive effects of HDAC on chromatin associated with myogenic genes</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Akt3</td>
<td>Inhibits AKT/mTOR signaling</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>miR-675-3p</td>
<td>Smad1/5/6</td>
<td>Inhibits TGF-β signaling</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cdc6</td>
<td>Promotes cell cycle arrest and differentiation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>miR-675-5p</td>
<td>Smad1/5/6</td>
<td>Inhibits TGF-β signaling to promote myogenesis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cdc6</td>
<td>Promotes cell cycle arrest and differentiation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>miR-199a-3p</td>
<td>Igf-1</td>
<td>Inhibition of AKT/mTOR Signaling</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pik3r1</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>mTOR</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>miR-155</td>
<td>Mef2c</td>
<td>Inhibits differentiation and promotes proliferation by suppressing Mef2c levels thereby not allowing its association with myogenic regulatory factors</td>
<td>66</td>
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<tr>
<td></td>
<td>miR-181</td>
<td>Hox-A11</td>
<td>Promotes upregulation of MyoD which is inhibited by Hox-A11</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>miR-23a</td>
<td>Myh 1, 2 and 4</td>
<td>Suppresses expression of contractile proteins required for the terminally differentiated phenotype</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>miR-148a</td>
<td>Rock1</td>
<td>Cytoskeleton stability</td>
<td>103</td>
</tr>
</tbody>
</table>

3.2. Differentiation

Relative to the limited number of studies investigating the function of miRNAs in myoblast proliferation, the role of miRNAs in regulating myogenic differentiation has been more fully explored. As would be expected, the initial studies focused on those miRNAs that are enriched in skeletal muscle, the myomiRs. Chen and colleagues (2006) demonstrated that miR-1 promoted differentiation by relieving chromatin-based repression of the myogenic regulatory factors through regulation of Hdac4 (histone deacetylase 4) expression (52). The same group went on to show that miR-1 and -206 restrict myogenic progenitor cell proliferation and promote differentiation by directly regulating expression of Pax7 (72). The regulation of Pax7 expression by miR-206 was more recently confirmed by Dey and coworkers, who further demonstrated that Pax7 expression was also subject to regulation by miR-486 (73). Another mechanism through which miR-206 promotes myogenic differentiation is by inducing myoblast to exit the cell cycle through repression of specific subunits of DNA polymerase α as well as by targeting Hdac4 (51, 74). Similarly, miR-1 has been shown to regulate cell cycle progression by targeting Cyclin D1, thereby promoting G1 cell cycle arrest (75). During myoblast fusion, both miR-1 and -206 have been shown to inhibit the translation of Gja1 (gap junction protein, alpha 1)/Connexin43 in order to block the formation of gap junctions (76). Although Connexin43 expression is necessary for myoblast fusion and muscle...
regeneration, its down-regulation is required for myotube survival (77, 78). Furthermore, it is likely that miR-1 and miR-206 regulate many other genes involved in the differentiation process, as manipulation of their levels during differentiation results in significant alterations in the transcriptome of C2C12 cells (79).

The role of miR-133 during myogenic differentiation appears to be more complex than miR-1/206. Early reports indicated that miR-133 possessed pro-proliferative properties through the regulation of Srf expression (52); however recent studies are challenging these findings, suggesting that miR-133 promotes differentiation similar to miR-1 and miR-206 (75, 80). Although these latest findings seem to be biologically more consistent (i.e., serving a similar function), given that miR-1 and miR-133 are derived from the same pri-miRNA transcript, Zhang et al. (2012) showed that miR-133, through p38-mediated signaling, inhibited proliferation by directly targeting the transcription factor Sp1 (trans-acting transcription factor 1), which in turn activates Cyclin D1 expression (75, 81). Similarly, a more recent study demonstrated that miR-133 promoted differentiation by down-regulating two members of the ERK1/2 signaling pathway, FGFR1 (fibroblast growth factor receptor 1) and PP2AC (protein phosphatase 2, catalytic subunit, alpha isozyme), which the authors showed were part of a pro-proliferation signaling cascade (80).

In addition to the muscle-enriched miRNAs, other miRNAs have been implicated in potentially playing a role in myogenic differentiation. Wong and coworkers (2008) showed that endogenous levels of miR-26a increased during myogenic differentiation and that over-expression of this miR-26a promoted the myogenic program (82). These authors reported that miR-26a regulated the expression of Ezh2 (enhancer of zeste homolog 2), a catalytic subunit of the polycomb repressive complex, thereby relieving the repressive histone modifications on multiple myogenic genes (82). MiR-26a has also been shown to down-regulate TGF-β signaling, a well-established inhibitor of myogenesis, by targeting two downstream effector genes of the pathway, Smad1 and Smad4 (83). Similarly, MyoD and myogenin are able to drive expression of miR-214, which also targets Ezh2, providing a feedback loop to promote the expression of genes required for differentiation (84). In addition, miR-214 can also promote myoblast cell cycle exit by repressing the proto-oncogene gene N-ras (neuroblastoma ras oncogene) expression along with potentially playing a role in specifying myogenic lineage (85, 86). MiR-322/424 and -503 have also been shown to promote cell cycle exit of myoblasts through the down-regulation of Cdc25A (cell division cycle 25A), the phosphatase responsible for removing the inhibitory phosphorylation on T14 and Y15 of CDK2 (cyclin-dependent kinase 2) (87).

MiR-29b/c is another non-muscle-specific microRNA that appears to play an important role in regulation of myogenesis (55, 74, 88-92). MiR-29 b/c was first identified as a potential factor involved in myogenesis through the identification of upstream binding sites for the Polycomb group protein, YY1 (yin yang 1) (88). YY1 is a downstream target of the NFκB (nuclear factor kappa B) signaling pathway that functions to inhibit myogenesis through repression of myogenic gene expression. Interestingly, YY1 itself is a target of miR-29 which provides a feedback loop in which increased expression of miR-29 serves to relieve the repressive effects of YY1 and promote myogenesis (88). It was later shown that miR-29 also targets another gene in this pathway, Rybp (Ring 1 and YY1-binding protein), which the authors demonstrate co-occupies myogenic loci along with YY1 (91). Therefore, it appears that a primary function of miR-29 is to promote myogenesis by down-regulating multiple targets in the NFκB signaling pathway. In addition to genes within the NFκB pathway, miR-29 also regulates the expression of Hdac4 and Akt3 to promote myogenic differentiation (74, 90). Not only does it appear that miR-29 is responsible for promoting myogenesis, but it may also play a role in preventing myoblasts from adopting an alternate cell fate (92). The canonical...
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TGF-β/Smad signaling pathway, a well-known pathway involved in fibrosis formation, appears to negatively regulate the expression of miR-29 thereby promoting the conversion of myoblasts to myofibroblasts (92). In support of the potential involvement of miR-29 in development of fibrosis, Wang and colleagues (2012) demonstrated that the loss of miR-29 may play a role in the development of a dystrophic phenotype (55).

MiR-181 has been shown to promote myogenic differentiation by targeting Hoxa11 (homeobox A11), a negative regulator of MyoD expression (93, 94). Inhibition of miR-181 decreased differentiation of C2C12 myoblasts; however, over-expression of miR-181 was not capable of inducing myogenic differentiation, leading the authors to conclude that miR-181 is necessary, but not sufficient, for myogenic differentiation (93). Recently, two novel microRNAs, miR-675-3p and miR-675-5p were identified within an exon of an imprinted long noncoding RNA, H19 (95). While H19 is normally not expressed in adult tissue, the authors eloquently demonstrated that not only is H19 expressed in skeletal muscle, but these two miRNAs are able to promote myogenic differentiation through the down-regulation of multiple factors, including Smad1, Smad5 and Cdc6 (cell division cycle 6) (95).

The Mef2 isoforms are transcription factors that act in conjunction with MRFs to regulate muscle differentiation (58). One aspect of Mef2 regulation of myogenesis is through the control of the tissue-specific expression of the bicistronic precursor RNA encoding miR-1-2 and 133a-1 (81). In addition, Mef2a was recently reported to control the expression of a large number of miRNAs located in the Gtl2-Dio3 locus through the regulation of an upstream promoter (96). In vitro, miR-155 has been shown to repress Mef2a expression thereby blocking myogenic differentiation (66). The Mef2c isoform was shown to be targeted by miR-27b, which is consistent with the idea that miR-27 promotes the proliferative state of myogenic cells (70, 97). Furthermore, through the regulation of Pax3, over-expression of miR-27b using a conditional transgene resulted in abnormal migration of Pax3+ progenitor cells along with premature differentiation (98). Recently a cardiac- and muscle-specific miRNA, miR-92b, was identified in Drosophila and found to be required for normal muscle development through its regulation of Mef2 (63). In addition, the canonical myomiRs miR-1 and -206 were reported to indirectly regulate Mef2c through repression of Notch3 expression, which under normal conditions antagonizes Mef2c expression to inhibit myogenic differentiation (64).

Finally, upon differentiation, myofibers begin to express contractile proteins, including multiple myosin heavy chain isoforms. Recently, miR-23a was shown to inhibit myogenic differentiation by targeting multiple adult fast myosin heavy chain genes, including Myh 1, 2 and 4 (99). In addition, muscle cells contain a highly structured cytoskeleton, with assembly of the cytoskeleton governed primarily through RhoA (ras homolog family member A) signaling (100). ROCK1 (Rho-associated, coiled-coil containing protein kinase 1) is a downstream target of RhoA, which acts to stabilize actin, and whose expression is required to be down-regulated to allow for terminal myoblast differentiation presumably to allow for cytoskeleton remodeling during myoblast fusions (101, 102). The down-regulation of Rock1 expression is partially controlled by miR-148a targeting; accordingly, inhibition of miR-148a activity by an antagonim impairs myogenic differentiation (103). Therefore, miRNAs not only control myogenic differentiation through the regulation of transcription factors but also through the direct regulation of structural proteins and cytoskeletal components that are required to give rise to the differentiated phenotype.

3.3. Quiescence

In vivo, myogenic stem cells or satellite cells are normally in a quiescent state following post-natal development. This makes studying their behavior difficult, and precludes the use of in vitro models to study the role of miRNAs in the regulation of this quiescent state. Only recently has evidence emerged demonstrating
that miRNAs are involved in maintaining quiescence in satellite cells (67, 68, 104). Using the conditional inactivation of Dicer in satellite cells, Cheung and coworkers showed that mature miRNAs were required to maintain satellite cell quiescence, as satellite cells that lacked a functional Dicer gene spontaneously exited the quiescent state (67). These same authors went on to demonstrate that miR-489 is a key miRNA involved in satellite cell quiescence due to its regulation of the proto-oncogene Dek, which normally acts to promote the proliferative expansion of myogenic progenitors (67). Another miRNA, miR-31, was found to regulate the expression of the myogenic regulatory factor Myf5 in quiescent satellite cells (68). Crist and coworkers (2012) reported that miR-31 and Myf5 are held in close proximity to one another as part of messenger ribonucleoprotein particles (mRNPs) (68). Upon satellite cell activation, these mRNPs rapidly dissociate and relieve the spatial constraint on miR-31 and Myf5, allowing for the rapid translation of the Myf5 protein (68). Furthermore, miRNA expression profiling of isolated quiescent satellite cells, revealed that additional miRNAs are down-regulated in comparison to proliferating and differentiating cells (104). These finding suggest that perhaps there are other miRNA required to maintain the quiescent state of satellite cells; however, whether the involvement of these miRNAs is direct or indirect, as well as their target genes, remains to be elucidated.

4. MANIPULATION OF MICRONAS EXPRESSION ON DE NOVO SKELETAL MUSCLE FORMATION

4.1. Development

Results from in vitro studies have greatly enhanced our understanding of the potential role that miRNAs have in myogenesis in vivo, though the biological significance in the context of a whole organism will undoubtedly be more complex. For example, mice that are null for individual muscle-specific miRNAs show no obvious skeletal muscle phenotype (37, 38, 45-49). The lack of a phenotype in these different miRNA null mice may in part reflect overlap in their seed sequences, as is the case with miR-1 and -206, or that individual genes may contain targets for multiple miRNAs. Regardless, it is clear that miRNAs are absolutely required for proper embryonic development, as germline Dicer knockout mice die in utero at day E7.5. (105). Furthermore, a more targeted approach has demonstrated that miRNAs are required for skeletal muscle development, as mice that have Dicer deleted specifically in skeletal muscle die perinatally and exhibit decreased muscle mass and abnormal myofiber morphology (106). The decrease in Dicer-/- muscle mass is first notable at E14.5 and becomes drastically reduced at E18.5. This loss in muscle mass is the result of hypoplasia and not hypotrophy; despite having less mass, there does not appear to be a change in fiber-type (106). Though somewhat speculative, this phenotype might reflect the loss of miR-1 expression, which in Drosophila has been shown to be necessary for skeletal muscle development and viability (42, 106). The importance of miR-1 in development was further illustrated by Chen and colleagues (2006), who introduced either miR-1 or miR-133 into Xenopus laevis embryo (52). The misexpression of either miR-1 or -133 affected cardiac and skeletal muscle formation, however miR-1 demonstrated a much more dramatic phenotype, with no development of cardiac tissue and highly disorganized somites (52). Surprisingly, either the loss of miR-133a-1 or miR-133a-2 or the over-expression of miR-133a1-2 in mice results in no overt skeletal muscle phenotype (53, 108). In contrast, the double-knockout of both miR-133a-1a and -133a-2 caused lethal septal defects in approximately 50% of the offspring with those surviving to adulthood developing dilated cardiomyopathy (53).

4.2. Regeneration

One of the defining aspects of skeletal muscle is the capacity to mount a robust regenerative response following extensive injury. Due to this ability to regenerate so effectively, another technique commonly employed to study the role of miRNAs on
Function of microRNA in skeletal muscle

Functional aspects of microRNA in skeletal muscle formation is through the use of an in vivo injury model. The reparative process requires muscle satellite cells to become activated, proliferate and then differentiate such that perturbation of miRNA levels during this process can provide a readout for their function in the context of a whole organism. Studies using regeneration as a model of myogenesis are critical for furthering our knowledge of miRNAs in skeletal muscle biology, as the complexity of factors that exist in vivo more than likely have a dramatic impact on the expression and function of numerous miRNAs. Various studies have provided evidence that muscle injury results in the altered expression of numerous miRNAs (93, 109), though a few studies have performed gain- and loss-of-function experiments to characterize the functional outcome with regards to regenerative capabilities (83, 98, 110). Crist and colleagues (2009) showed that inhibition of miR-27 delayed muscle regeneration by repressing Pax3 expression in satellite cells (98). Conversely, Nakasa et al. (2010) reported enhanced muscle regeneration following a laceration injury by injection of exogenous myomiRs miR-1, -133 and -206 (111). These authors showed that this myomiR concoction caused increased expression of MyoD, myogenin and Pax7 while concomitantly decreasing expression of myostatin (111). Another study confirmed the regulation of Pax7 expression and satellite cell proliferation by miR-1 and -206 during muscle regeneration, though the authors did not investigate the effect of this regulation on the regeneration process (72). A later report did however demonstrate that loss of miR-206 expression exacerbated injury-induced regeneration through defective differentiation of satellite cells, mostly likely due to the inability to down-regulate Pax7 expression (46). Furthermore, miR-206 has been recently shown to target the Hmgb3 (High Mobility Group Box 3) gene during regeneration (112), which is known to be involved in stem cell self-renewal and differentiation (113). Collectively, the results from these studies indicated that miR-1 and -206 are required for skeletal muscle regeneration, with a primary function to insure the proper down-regulation of genes involved in the differentiation process of satellite cells.

MiRNAs other than myomiRs have been shown to regulate satellite cell proliferation and differentiation by altering TGF-β signaling pathway. MiR-26a expression is rapidly down-regulated following muscle injury but then slowly increases a few days following the injury (83). Dey and colleagues (2012) demonstrated that miR-26a is required to suppress TGF-β signaling during differentiation through the regulation of SMAD1 and 4, whereas muscles with reduced miR-26a levels showed a delayed regenerative response (83). This same group recently identified two additional novel miRNAs, miR-675-3p and miR-675-5p, which are derived from the H19 long non-coding RNA and are required for proper muscle regeneration through by inhibiting TGF-β signaling (95). Restricting TGF-β signaling further promotes myogenic differentiation by permitting an increase in the expression of the pro-differentiation miRNAs, miR-29 and -206 (74). Together, the findings of these studies confirm the numerous in vitro studies examining the central role that miRNAs have in the regulation of myogenic differentiation.

Recently, the well-studied mTOR (mechanical target of rapamycin) pathway has been implicated during skeletal muscle regeneration, as inhibition of this pathway impairs injury-induced regeneration (114). One mechanism through which mTOR is thought to regulate regeneration is by affecting the expression of Igf-II (insulin-like growth factor II) (114). Part of this regulation appears to involve miR-125b, which has been shown to be negatively regulated by mTOR (110). Therefore, miR-125b acts as a negative regulator of muscle regeneration through its suppression of Igf-II expression, and furthermore, decreasing miR-125b levels during regeneration ameliorated this process (110). In addition, mTOR activation also increases the expression of miR-1 which then controls satellite cell activity as previously outlined (115). AKT/IRS1 (insulin receptor substrate-1) signaling lies upstream of the mTOR complex and is involved
in regeneration. Recently, Alexander and colleagues (2011) demonstrated that during regeneration AKT signaling is regulated by miR-486 (116). Normally, miR-486 is induced during regeneration allowing for activation of AKT through the down-regulation of PTEN (116), a well-characterized negative regulator of AKT signaling. Inappropriate over-expression of miR-486 resulted in abnormal regeneration, presumably through the misregulation of many target genes (116). Another miRNA, miR-199a-3p, was recently shown to regulate multiple targets within the AKT/mTOR signaling pathway in vitro; however, the importance of miR-199a-3p in the regulation of skeletal muscle regeneration remains unknown (117).

These findings indicate that components of the AKT/mTOR signaling pathway are subject to regulation by both muscle-specific and non-muscle-specific miRNAs.

4.3. Innervation

The functionality of skeletal muscle requires innervation by the central nervous system in order to form muscle motor units. Dysfunctional or loss of innervation results in multiple muscular disorders, as will be discussed in later sections. Therefore, the formation and maintenance of the neuromuscular junction (NMJ) is a critical step in the formation of mature skeletal muscle, of which microRNAs have shown to perform an integral role. Specifically, it appears that microRNAs play a role in synapse formation (118, 119), NMJ sensitivity (120-122), and reinnervation following injury (47, 123). During development in Drosophila, let-7 and miR-125 control the maturation of the NMJ through targeting of the abrupt gene (118, 119). Originally described in C. elegans, miR-1 directly regulates the expression acetylcholine (Ach) receptor subunits, along with pre-synaptic Ach release in a MEF-2-dependent mechanism (120). These findings suggest that miR-1 is involved in regulation Ach sensitivity within the NMJ. MicroRNA regulation of neurotransmitter release has also been described in Drosophila melanogaster, where the miR-310 cluster negatively regulated synaptic function by targeting Khc-73, a kinesin gene required for normal synaptic function (122). Similarly, miR-124 mutants demonstrate aberrant NMJ activity, with miR-124 serving to limit synaptic activity (121). Furthermore, miR-8 negatively regulates the expansion if pre-synaptic terminals (124), both through the regulation of Ena (Enabled) (125) and Wg (wingless) (126).

In mammals, miR-206, but not miR-133b, is required for reinnervation of muscle fibers following injury (47, 123). Recently, miR-206 was shown to regulate Bdnf (brain-derived neurotrophic factor), a factor known to be involved in regulation of innervating motor neurons, in differentiating myoblasts (127). However, it remains to be determined whether...
this regulation is important for the reinnervation process. Further research is required to identify additional targets of miR-206 within the NMJ in order to elucidate the underlying mechanism by which miR-206 controls reinnervation. Collectively, these findings suggest a critical role for microRNAs during NMJ formation, with further research required to whether they play a role in regulating the maintenance and activity of motor units during periods of muscle maintenance, neuromuscular adaptation and aging.

5. MICRONAS IN RESPONSE TO MUSCULAR ACTIVITY

It is clear that miRNAs are required for muscle development and regeneration; however their role in normal muscle maintenance and adaptation during adulthood has not been well characterized. In order to stimulate muscle adaptation in adulthood, there has to be increased demand, either mechanical or metabolic, placed on the muscle. Commonly, this is achieved through various modes of physical activity, such as resistance or aerobic exercise. For simplicity sake, this section will focus on literature that has shown changes in miRNA levels to either increased mechanical (i.e. resistance exercise) or metabolic (i.e. aerobic exercise) demand. Finally, we will examine emerging literature showing that miRNAs may be released from tissue during physical activity, potentially to act as signaling factors to peripheral tissues.

5.1. miRNAs in response to increased mechanical demand

Our group was the first to demonstrate that miRNA levels could be modulated by changes in mechanical demand (128). We utilized a mouse model in which mechanical overload is placed on the plantaris muscle though the surgical removal of two synergist muscles, the gastrocnemius and soleus. Following 7 days of mechanical overload, the plantaris showed an ~50% decrease in mature miR-1 and -133 levels, suggesting that perhaps this down-regulation is required for muscle adaptation, including muscular hypertrophy (128). In agreement with the mouse data, in humans an acute bout of resistance exercise results in a significant decrease in miR-1 expression in skeletal muscle (129), perhaps in order to potentiate activation of the IGF1/AKT signaling cascade given that miR-1 has been shown to target Igf-1 and the Igf-1R (Igf-1 receptor) (130). In addition, there is evidence to suggest that the magnitude of change in miRNA expression following resistance exercise training is a predictor of how well a person will respond to the exercise (32). A recent study by Davidsen and colleagues examined whether changes in miRNA expression in the vastus lateralis muscle corresponded to differences in muscle hypertrophy. A cohort of young, adult males underwent 12 weeks of resistance training with post-hoc analysis separating the group into “low-responders” and “high-responders” based on each subject’s change in lean body mass. Twenty-one miRNAs were profiled, all showing no significant change in the high-responder group, whereas the low-responder group showed a significant change in miR-451 (increase) and miR-378 (decrease), with a downward trend for miR-26a and miR-29a. Furthermore, the change in miR-378 expression showed a significant correlation \( R^2=0.51 \) to the change in lean body mass, leading the authors to speculate that perhaps decreases in miR-378 expression levels may hinder muscle accretion that accompanies resistance exercise (32). The link between miR-378 levels and muscle adaptation has not been well studied; however miR-378 has been shown to be positively regulated by MyoD, so perhaps miR-378 regulates a MyoD-dependent mechanism involved in skeletal muscle adaptation to exercise (131).

5.2. MiRNAs in response to increased metabolic demand

Skeletal muscle is a large consumer of oxygen and is one of the primary tissues that determine basal metabolic rate. In response to increased metabolic demand, such as during aerobic exercise, skeletal muscle has the ability to adapt to this increase
through mechanisms that include increasing capillarization, mitochondrial biogenesis, and increased metabolic enzyme content. Emerging evidence, while still correlative, suggest that metabolic changes in the muscle alter miRNA levels, which then may contribute to promoting adaptation. The first indication that miRNAs may be involved in regulating the metabolic phenotype came from work by van Rooji and coworkers (2009) who identified two miRNAs, miR-208b and miR-499, encoded within slow-twitch, type I myosin genes Myh7 and Myh7b, respectively (37). The targeted deletion of miR-208b or miR-499 revealed that these two miRNAs were required to establish the slow-twitch fiber phenotype as null mice for either miRNA resulted in a muscle with significantly more fast-twitch fibers. Consistent with this finding, these knockout mice exhibited reduced exercise capacity when subjected to forced running (37).

Safdar and colleagues (2009) were the first to examine whether an acute bout of endurance exercise in mice was sufficient to modulate specific miRNAs known to target common genes associated with exercise adaptation (132). Using this candidate approach, they demonstrated that the exercise bout significantly increased the expression of miR-181, miR-1 and miR-107 by 37%, 40% and 56%, respectively, and reduced miR-23 expression by 84%. These changes in miRNA expression were associated with increased expression of the miR-23 target, Pgc-1α (peroxisome proliferator-activated receptor gamma, coactivator 1 alpha), as well as downstream targets of PGC-1α involved in mitochondrial biogenesis (132). In addition, Pgc-1α has been shown to be targeted by miR-696, another miRNA that is down-regulated in response to endurance exercise (133). Further evidence suggesting that miRNA levels may control mitochondrial biogenesis was observed by Yamamoto et al. (2012), who demonstrated that a novel miRNA, miR-494 is suppressed following endurance exercise. This corresponded to a concomitant increase in gene targets involved mitochondrial biogenesis, including Pgc-1α, mtTFA (mitochondrial transcription factor A) and Foxj3 (forkhead box J3) (134). In addition to mitochondrial biogenesis, miRNAs may be involved in regulating adaptations involving oxygen deliver to the muscle via increased capillary density. Fernandes and coworkers (2012) examined miRNA levels in the soleus muscle of rats following swim training and found that miR-16 levels significantly decreased, paralleled by an increase in protein levels for VEGF (vascular endothelial growth factor) and its respective type 2 receptor (135). Vegfa has been validated as a target for miR-16 (136), and expression of Vegf is required for exercise-induced increased in capillary density in response to exercise (137). Therefore, these alterations within the VEGF pathway were thought to modulate angiogenesis, as the authors observed a concomitant increase in capillarization within the muscle (135).

In untrained human participants, 60 minutes of endurance exercise was reported to increase the expression of miR-1 and miR-133 in the vastus lateralis muscle, however, following 12 weeks of training the resting levels of miR-1, miR-133a, miR-133b and miR-206 were lower than pre-training (138). Interestingly, the changes observed following the acute 60 minute bout pre-training was abolished post-training (138), suggesting that miRNA levels in response to exercise are sensitive to training status. Similarly, Russell and colleagues (2013) reported increased miR-1, -133a, -133-b and -181a expression coupled with a decrease in miR-9, -23a, -23b and -31 expression following an acute bout of exercise (139). These same authors found that following 10 days of training miR-1 and miR-31 expression was still increased and decreased, respectively, and that training had induced an increase in miR-29b expression. Further characterization of miR-31 revealed that there were negative correlations associated with miR-31 levels and HDAC4 and NRF1 (nuclear respiratory factor 1) levels, both predicated targets of miR-31 that the authors went on to validate (139). These findings support the results from animal studies, which showed the
same pattern of expression for miR-1, -181 and -23 in response to endurance exercise (132). Employing a more comprehensive microarray approach, Keller et al. (2011), demonstrated that 6 weeks of cycling significantly decreased the expression of miR-1 and miR-133, along with miR-101 and miR-455 (140).

5.3. Physical activity and circulating microRNA

An emerging area of interest in the miRNA field is the presence of miRNAs in the circulation and their potential to act as signaling molecules to affect peripheral tissues. With respect to physical activity, since skeletal muscle is the predominant tissue that is being activated, it would seem plausible that it may be the primary source contributing to altered miRNA levels in the circulation, however it cannot be ruled out that additional tissues are not involved. Radom-Aizik and colleagues (2010) were the first to look at the effects of acute exercise on changes in miRNA levels in the circulation, however they focused on miRNAs specifically within neutrophils (141). They followed up this work by examining other cell types in the circulation, including mononuclear cells (142) and natural killer cells (143). Baggish and colleagues (2011) investigated miRNAs in plasma from human subjects, choosing not to isolate a specific cell type, in response to either a bout of aerobic exercise or following a chronic training period (144). They observed that an acute bout of endurance exercise produced an increase in circulating levels of miR-146a, miR-222, miR-21 and miR-221. Furthermore, following the 90 day training period, the basal level of these miRNAs remained elevated in addition to miR-20a. Interestingly, even after the training period, circulating levels of these miRNAs still showed the transient increase following an acute bout of exercise (144). It should be noted that the authors utilized a candidate approach to focus their search on miRNA known to regulate genes involved in exercise adaptation and only included one muscle-specific (miR-133a) miRNA. With this in mind, Aoi et al. (2013) looked specifically at muscle-enriched miRNA (miR-1, miR-133a, miR-133b, miR-206, miR-208b, miR-486 and miR-499) and whether their expression level changed in response to either acute or chronic aerobic exercise (145). As would be expected, these miRNAs were found at extremely low levels in the plasma, however miR-486 was the most abundant and showed a significant change with aerobic exercise, decreasing with both acute and chronic exercise. The mechanism or the significance of this decrease is unclear, although miR-486 has been shown to target an insulin signaling (PTEN), leading the authors to postulate that it may be involved in glucose and metabolic regulation during exercise (145).

Besides the type of exercise, the exercise modality may also influence the miRNAs found in the circulation, as resistance exercise has been shown to cause minor changes in miRNA levels at various time points following the exercise bout (146). Similarly, exercise bouts that primarily involve concentric muscle contractions show a differential response when compared to eccentric-based exercise (147). Eccentric-based exercise, which typically results in muscle damage, demonstrates a greater increase in the muscle-specific miRNAs which therefore may serve as useful biomarkers for muscle damage (147, 148). The idea that circulating miRNAs may serve as biomarkers has been around for some time in the cancer field, however only recently have groups been examining whether they have any relevance with respect to exercise capacity (149-151). Recently, a large cross-sectional study sought to determine if there was any relationship between circulating miRNAs levels and aerobic capacity, by stratifying individuals based on high or low maximal oxygen uptake (VO₂ max) (149). When examining results from both men and women, the authors found that individuals with low VO₂ max had significantly higher levels of miR-210 and miR-222, 30% and 20% respectively, when compared to participants with high VO₂ max. In just men, miR-21 was also significantly elevated by 20% in individuals with low VO₂ max (149). Similarly, Mooren et al. (2014) sought to determine whether a specific subset of miRNAs (miR-1, -133, -206, -499, -208b,
-21, and -155) correlated to aerobic capacity in trained marathon runners (151). Interestingly, only myomiRs miR-1, -133 and -206 demonstrated a significant positive correlation to an individual's VO\(_2\) max (151). Recently, Nielsen and colleagues (2014) set out to define the temporal pattern of miRNA expression in the circulation following an acute bout and then in response to chronic training (152). These authors found that immediately post-exercise there was a rapid overall decrease in a subset of miRNAs, however in the hours following exercise (1-3) miRNA levels were generally increased, including miR-1, -133a and -133b, which is consistent with what others have shown at this time point (139). In addition, following chronic training, it appears that the majority of miRNAs were down-regulated, including miR-133, miR-29b and miR-92, similar to what has been reported in skeletal muscle in response to training (138, 140).

Collectively, the findings of these studies raise some intriguing questions regarding the roles of muscle-enriched miRNAs during exercise adaptation, specifically aerobic exercise. Acutely, an exercise bout increases the levels of muscle-specific miRNAs, while following training their levels return to baseline in the muscle, however this may be due to increased secretion into the circulation. This hypothetical model is summarized in Figure 2. Future investigations should examine miRNA levels both in skeletal muscle and in the circulation at multiple time points during exercise adaptation. Perhaps miRNA-mediated regulation may be one of the underlying mechanisms by which exercise produces beneficial effects on peripheral organs. This will no doubt be an exciting new area of research in the years to come.

6. MICRORNA IN SKELETAL MUSCLE DISORDER AND ATROPHY

Skeletal muscle disorder and atrophy can be divided into three major categories: primary muscular disorders, secondary muscular disorders and aging sarcopenia (153). Primary muscular disorders are the consequence of a disease that directly affects skeletal muscle, such as muscular dystrophies, inflammatory myopathies and congenital myopathies.
Secondary muscular disorders are either disease-related, which include for example diabetes mellitus and chronic kidney disease, the result of neurodegenerative diseases, such as amyotrophic lateral sclerosis, or are a consequence of muscle disease, resulting from bed-rest, immobilization or space flight. This section of the review will discuss the current understanding of miRNA regulation and its potential role in skeletal muscle dysfunction and atrophy. We will also discuss the miRNAs involved in rhabdomyosarcoma, a predominantly pediatric sarcoma derived from skeletal muscle progenitor cells.

6.1. Primary muscular disorder

Although primary muscular disorders are typically classified according to their clinical and pathological manifestations, these pathologies are almost always characterized by progressive skeletal muscle weakness and wasting, resulting in impaired locomotion (154). The major primary muscular disorders include several dystrophies such as Duchenne muscular dystrophy (DMD), myotonic dystrophy type 1 and 2, Becker muscular dystrophy (BMD), facioscapulohumeral muscular dystrophy (FSHD) and limb-girdle muscular dystrophy type 2A and 2B, as well as other myopathies, such as Miyoshi myopathy, nemaline myopathy, and the three inflammatory myopathies dermatomyositis, inclusion body myositis and polymyositis. Eisenberg and colleagues (2007) showed in a microarray study that 185 miRNAs are dysregulated in at least one of these ten primary muscular disorders, and the major portion of the dysregulated miRNAs was up-regulated (155). Among these miRNAs, miR-146b, miR-221, miR-155, miR-214, miR-222 and miR-34a were consistently up-regulated in at least nine of the ten different myopathies; moreover, most of the miRNAs have a similar pattern of expression in the diseases in which they were identified as differentially expressed in comparison to healthy human skeletal muscle. The study by Eisenberg and colleagues was the first to provide strong evidence that dysregulation of miRNA expression is a common feature of primary muscular disorders (See Table 2).

6.1.1. Duchenne muscular dystrophy

Numerous studies have used animal models to better understand the potential role of miRNAs in dystrophic diseases, as exemplified by the mdx mouse. The mdx mouse is a well-established model of DMD, the most common and severe form of dystrophy characterized by the absence of the cytoskeleton protein dystrophin (55, 156-160). We have previously shown that the muscle-specific miRNA (myomiR) miR-206 was up-regulated in the diaphragm of the mdx mice, the most severely affected muscle in these dystrophic animals (159). The increase in miR-206 expression in skeletal muscle of mdx mice was confirmed by others (158, 160), while the loss of miR-206 accelerates and exacerbates the dystrophic phenotype in this DMD model (46). In addition to miR-206, ten other miRNAs were found to be dysregulated in both the mdx mouse and DMD patients (158). These authors proposed three classes of DMD-signature miRNAs, including regeneration miRNAs (up-regulated miRNAs: miR-31, miR-34c, miR-206, miR-335, miR-449, and miR-494), degenerative miRNAs (down-regulated miRNAs: miR-1, miR-29c, miR-135a) and inflammatory miRNAs (up-regulated miRNAs: miR-222 and miR-223). Interestingly, they reported that both administration of a histone deacetylase inhibitor and the overexpression of endothelial nitric oxide synthase rescued the dystrophic phenotype of the mdx mouse apparently by normalizing miRNA expression (158).

The findings from other studies have identified additional miRNAs that may offer alternative therapeutic strategies to improve the dystrophic phenotype. Cacchiarelli and coworkers found that expression of miR-31 was significantly increased in skeletal muscle and isolated myoblasts from DMD patients and was able to repress dystrophin expression (158, 161). This latter study showed that treating human myoblasts with exon-skipping oligonucleotide resulted in miR-31 inhibition, rescuing dystrophin expression. Moreover, administration of miR-29, a miRNA down-regulated in skeletal muscle of DMD patients (55, 158) improved dystrophy
Table 2. MicroRNA dysregulation associated with muscle disorders

<table>
<thead>
<tr>
<th>Category</th>
<th>Family</th>
<th>Disease/disorder</th>
<th>miRNA expression</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary muscular disorder</td>
<td>Duchenne muscular dystrophy</td>
<td></td>
<td>↑ of 39 miRNAs and ↓ of 23 miRNAs; ↑ miR-199a-5p; ↓ miR-466; ↑ of 8 miRNAs (including miR-206 and miR-31), ↓ of 3 miRNAs (including miR-1); ↑ miR-1, miR-206 and miR-133 in serum of DMD children; ↑ miR-21</td>
<td>155; 156; 116; 158; 220; 157</td>
</tr>
<tr>
<td></td>
<td>Myotonic dystrophy (type 1 and 2)</td>
<td></td>
<td>↑ miR-206 in most of the DM1 patients, no effect on miR-1; ↑ miR-1 and miR-35, ↓ miR-29b, miR-29c and miR-33 in DM1 patients; ↓ miR-1, miR-7 and miR-10a in DM1 patients; ↑ of 12 miRNAs (including miR-133b), ↓ of 8 miRNAs (including 133a) in DM2 patients</td>
<td>163; 162; 164; 165</td>
</tr>
<tr>
<td></td>
<td>Becker muscular dystrophy (BMD)</td>
<td></td>
<td>↑ miR-146b and miR-221</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>Facioscapulohumeral muscular dystrophy (FSHD)</td>
<td></td>
<td>↑ of 62 miRNAs; ↑ of 21 miRNAs (including miR-1, miR-33a, miR-133b and miR-206), ↓ 8 miRNAs in primary myoblasts of FSHD patients; ↑ of 2 miRNAs, ↓ of 6 miRNAs in primary myoblasts of FSHD patients</td>
<td>155; 167; 168</td>
</tr>
<tr>
<td></td>
<td>Limb-girdle MD type 2A and 2B</td>
<td></td>
<td>↑ of 88 (2A) and 87 (2B) miRNAs, ↓ of 4 (2A) and 5 (2B) miRNAs</td>
<td>155</td>
</tr>
<tr>
<td>Myopathy</td>
<td>Miyoshi myopathy</td>
<td></td>
<td>↑ of 68 miRNAs, ↓ of 5 miRNAs</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>Nemaline myopathy</td>
<td></td>
<td>↑ of 1 of 130 miRNAs, ↓ of 13 miRNAs</td>
<td>155</td>
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<td></td>
<td>Polymyositis</td>
<td></td>
<td>↑ of 37 miRNAs, ↓ miR-30a-3p; ↓ miR-1, miR133a and miR-133b</td>
<td>155; 172</td>
</tr>
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<td></td>
<td>Dermatomyositis</td>
<td></td>
<td>↑ of 35 miRNAs, ↓ of 2 miRNAs; ↓ miR-1, miR133a, miR-133b and miR-206; ↓ miR-126 in juvenile DM patients with short duration of untreated disease</td>
<td>155; 172; 173</td>
</tr>
<tr>
<td></td>
<td>Inclusion body myositis</td>
<td></td>
<td>↑ of 20 miRNAs, ↓ of 2 miRNAs; ↓ miR-1, miR133a and miR-133b</td>
<td>155; 172</td>
</tr>
<tr>
<td>Secondary muscular disorder</td>
<td>Neurodegenerative disease</td>
<td>Amyotrophic lateral sclerosis</td>
<td>↑ miR-206; ↑ miR-23a, miR-29b, miR-206 and miR-455</td>
<td>176; 175</td>
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<tr>
<td></td>
<td></td>
<td>CKD&lt;sup&gt;5&lt;/sup&gt;</td>
<td>↓ miR-486 in CKD mice; ↑ of 5 miRNAs (including miR-29a and miR-29b) in CKD mice</td>
<td>189; 89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diabetes mellitus</td>
<td>↓ miR-23a in diabetic rats; ↑ Let-7a and Let-7d in T2DM patients; ↑ of 10 miRNAs (including miR-29a and miR-29b) in T2DM patients</td>
<td>179; 181; 183; 180; 185</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COPD&lt;sup&gt;7&lt;/sup&gt;</td>
<td>↓ miR-1; ↑ miR-1, miR-133, miR-206 and miR-499 in plasma of COPD patients</td>
<td>190; 191</td>
</tr>
<tr>
<td></td>
<td>Disuse-related</td>
<td>Bedrest</td>
<td>↓ of 13 miRNAs (including miR-206 and miR-23a), ↑ of 2 miRNAs; ↓ miR-1 and miR-133a</td>
<td>201; 202</td>
</tr>
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<td></td>
<td></td>
<td>Immobilization</td>
<td>↓ miR-680, miR-696, miR-705 and miR-762 in mice</td>
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<td></td>
<td></td>
<td>Spaceflight</td>
<td>↓ miR-206 in mice</td>
<td>199</td>
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<td></td>
<td>Hindlimb suspension</td>
<td>↓ miR-107, miR-221, miR-499 and miR-208b in rats</td>
<td>200</td>
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(Contd...)
Function of microRNA in skeletal muscle

Table 2. Contd...

<table>
<thead>
<tr>
<th>Category</th>
<th>Family</th>
<th>Disease/disorder</th>
<th>miRNA expression</th>
<th>Reference</th>
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<td>Sarcopenia</td>
<td></td>
<td>No ↓ miR-1 in response to resistance exercise combined with EAA ingestion; ↑ Let-b and Let-e; ↓ of 36 miRNAs, ↑ of 21 miRNAs in mice; ↓ of 30 miRNAs, ↑ of 5 miRNAs in rhesus monkey</td>
<td>129; 210; 206; 207</td>
<td></td>
</tr>
<tr>
<td>Rhabdomyosarcoma (RMS)</td>
<td></td>
<td>Suppression of miR-1 and miR-206; ↓ miR-29b and miR-1; ↓ miR-1, miR-206, miR-133a and miR-133b; ↑ miR-301, ↓ miR-27a and miR-26a; ↑ miR-183; ↓ miR-203</td>
<td>214; 88; 216; 218; 219; 217</td>
<td></td>
</tr>
</tbody>
</table>

1↑, upregulation; 2↓, down-regulation; 3DM1, myotonic dystrophy type 1; 4DM2, myotonic dystrophy type 2; 5CKD, chronic kidney disease; 6T2DM, type 2-diabetes mellitus; 7COPD, chronic obstructive pulmonary disease; 8EAA, essential amino acids.

pathology by promoting regeneration and inhibiting fibrogenesis in mdx mice (55). An increased expression of miR-21 was also observed in DMD patients and in seven other primary muscular disorders (155, 157) and silencing this miRNA was shown to improve muscle dystrophy by reversing fibrosis in mdx mice (157). The expression of the muscle-enriched miR-486 was reduced in patients with DMD, as well as in mdx mice (116, 155). This miRNA was shown to alter cell cycle kinetics and the regeneration response following injury (116), suggesting that it may not be a relevant therapeutic strategy to ameliorate dystrophic disorders.

6.1.2. Myotonic dystrophy type 1 (DM1) and 2 (DM2)

DM1, the most frequent autosomal-dominant dystrophy in adults, is caused by the expansion of CTG repeats in the 3’ untranslated region of the Dmpk (dystrophia myotonica-protein kinase) gene. Some studies have reported in muscle biopsies of DM1 patients an over-expression of miR-206, miR-210, and miR-335 whereas miR-29b, miR-29c, miR-33, miR-7 and miR-10a were down-regulated (162-164). The dysregulation of miR-206, miR-29b and miR-29c is consistent with the changes observed in DMD patients (55, 158), suggesting that these miRNAs may have a role in muscle dysfunction related to these pathologies. An altered cellular distribution of the myomiR miR-206, miR-1 and miR-133b was observed in skeletal muscle of patients affected by DM1, while the changes in miR-1 expression remains controversial, as some studies reported either no change (163), a decrease (164) or an increase (162) in its expression. A recent study, using a fly model of DM1, showed that miR-10 over-expression increased lifespan (164), suggesting that this miRNA may be a relevant therapeutic target in DM1 patients.

DM2 displays milder clinical symptoms than DM1 and results from a tretanucleotidic repetition (CCTG)$_n$ in the first intron of the CCHC-type zinc finger, nucleic acid binding protein (Cnbp) gene. Expression profiling of miRNA in muscle from DM2 patients revealed a dysregulation of 20 miRNAs, including the up-regulation of miR-133b and the down-regulation of miR-133a, while most differentially expressed miRNAs did not share a similar pattern of expression with the skeletal muscle of DM1 subjects (165).

6.1.3. Facioscapulohumeral dystrophy (FSHD)

FSHD is an autosomal dominant disorder linked to the deletion of the D4Z4 repeats in the 4q35 subtelomeric region and most genes identified in this region are over-expressed in FSHD myoblasts, including the double homeobox genes Dux4 and Dux4c (166). A recent study reported an increased expression of 21 miRNAs in myoblasts of FSDH patients, including the myomiRs miR-1, miR-133a, miR-133b and miR-206, as well as a decreased
expression of eight miRNAs (167). These authors reported that the expression of 12 of the up-regulated miRNAs was also increased in cells transfected with DUX4C-expressing plasmid, suggesting that Dux4c regulates the expression of these miRNAs in FSHD patients. Interestingly, miR-107, miR-152 and miR-15a were consistently up-regulated in both myoblasts (167) and skeletal muscle (155, 167) of FSHD patients; however, several dysregulated miRNAs showed the opposite pattern of expression in these two studies. A recent study reported that miR-411 was up-regulated in myoblasts of FSHD patients (168). These authors proposed that miR-411 could be involved in the myogenic defect observed in FSDH myoblasts (169), by blunting YAF2 (YY1 associated factor 2) expression, thereby leading to the up-regulation of YY1, a negative regulator of myogenesis. In addition, an up-regulation of YY1 was observed in immortalized human myoblasts over-expressing DUX4 (170). Although it remains to be elucidated, Dux4 gene may have a potential role in the up-regulation of YY1 and the myogenesis defects observed in FSHD, through the dysregulation of miR-411.

6.1.4. Inflammatory myopathies

Inflammatory myopathies consist of a family of autoimmune/degenerative muscle diseases, including dermatomyositis, polymyositis and inclusion body myositis. Although these diseases differ in their clinical features, they share characteristics of chronic muscle inflammation and dysfunction (171). In their microarray study, Eisenberg and colleagues showed that the regulation of miRNA expression share some similarities in these three inflammatory diseases, as evidenced by the common up-regulation of eight miRNAs (miR146b, miR-155, miR-21, miR-34a, miR-221, miR-214 and miR-222) (155). Recently, a decreased expression of the myomiRs miR-1, miR-133a and miR-133b was observed in skeletal muscle in the three subtypes of inflammatory myopathies, while miR-206 expression was only reduced in dermatomyositis patients (172). Tnf-α (tumor necrosis factor α) expression was inversely correlated with the reduced expression of these myomiRs in these inflammatory myopathies, consistent with the ability of TNF-α to repress the expression of these myogenic miRNAs and impair differentiation of C2C12 myoblasts (172). Altogether, these results suggest that TNF-α contributes to the degenerative pathology of these inflammatory myopathies through the down-regulation of myomiRs. In addition, a decreased expression of miR-126 was observed in muscle of juvenile dermatomyositis patients with short duration of untreated disease, compared to patients with long duration of untreated disease and control subjects (173). Interestingly, this miRNA dysregulation was associated with the up-regulation of its predicted target Vcam-1 (vascular cell adhesion molecule 1), an adhesion factor involved in inflammation and tissue damage. This finding suggests a new potential mechanism involved in the juvenile dermatomyositis disease pathology.

6.2. Secondary muscular disorder

6.2.1. Amyotrophic lateral sclerosis (ALS)

ALS is a rapidly progressive neurodegenerative disorder affecting motor neurons, and leading to muscle denervation, atrophy, paralysis and ultimately death. Several studies have investigated the regulation of miRNAs in the central nervous system of ALS patients (19, 174) but information is limited in skeletal muscle. A recent study showed that miR-206 was dramatically up-regulated in skeletal muscle in a mouse model of ALS, while deficiency of miR-206 in this animal model accelerated the progression of the disease (47). These authors demonstrated that miR-206 promotes regeneration of neuromuscular synapses after nerve injury, most probably through the inactivation of Hdac4, suggesting that miR-206 could slow ALS progression. The increased expression of miR-206 was confirmed in ALS patients, but the changes remained moderate compared to those observed in the ALS mouse model (175, 176). Recently, Bruneteau and colleagues (2013) investigated whether the miR-206/Hdac4 pathway plays a role in the compensatory
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muscle reinnervation in ALS patients (176). They showed that Hdac4 up-regulation was higher in patients with rapidly progressive ALS compared to long-term ALS survivors, while the moderate increase in miR-206 expression were similar between these two groups. Although it remains to be tested, a therapeutic approach causing an increase in miR-206 expression in skeletal muscle may be beneficial to repress Hdac4 expression and reduce disease progression in ALS patients. In addition to miR-206, miR-23a, miR-29b and miR-455 were also up-regulated in skeletal muscle of ALS patients (175). Interestingly, these authors showed that miR-23a represses the expression of Pgc-1α, a master regulator of mitochondrial function that is impaired in ALS patients. These finding suggests that miR-23a could be involved in the skeletal muscle mitochondria dysfunction in ALS patients.

6.2.2. Disease-related muscular disorder

6.2.2.1. Diabetes mellitus

Diabetes mellitus is a metabolic disease characterized by the presence of a hyperglycemic state resulting from impairments in insulin release and/or function. Skeletal muscle is the primary site of post-prandial glucose intake, and muscle atrophy and metabolic perturbations commonly observed in both type 1 (T1DM) and 2 (T2DM) diabetic patients (177). Over the last five years, cumulative evidence has showed that miRNAs are involved in skeletal muscle dysfunction associated with diabetes mellitus (178). A decreased expression of miR-23a, a miRNA reported to repress the expression of Pgc-1α, a master regulator of mitochondrial function that is impaired in ALS patients. These finding suggests that miR-23a could be involved in the skeletal muscle mitochondria dysfunction in ALS patients.

6.2.2.2. Chronic kidney disease (CKD)

miRNAs were recently suggested to have a role in skeletal muscle wasting associated with CKD (188). Profiling of miRNA expression in skeletal muscle of mice with CKD revealed a dysregulation of 12 miRNAs (89). Among these miRNAs, miR-29a and miR-29b were down-regulated, and may contribute to the impaired muscle differentiation associated with CKD through the activation of YY1. A decrease in the expression of miR-486 was observed in this CKD model, with miR-486 over-expression blunting the muscle atrophy
associated with this model by blocking the activation of the FOXO1 (forkhead transcription factor O1)/atrogene pathway (189). Currently, the regulation of miRNA expression remains to be investigated in skeletal muscle of patients with CKD.

### 6.2.2.3. Chronic obstructive pulmonary disease (COPD)

COPD is another disease associated with skeletal muscle wasting and dysfunction. Analysis of miRNA expression in the skeletal muscle of COPD patients revealed a decreased expression of miR-1, whereas no change in miR-133 and miR-206 expression was observed (190). The decreased expression of miR-1 was associated with an increased level of Igf-I mRNA and HDAC4 protein, two validated miR-1 targets. Assessment of circulating myomiRs revealed an increased level of miR-1, miR-133, miR-206, and miR-499 in the plasma of the COPD patients (191).

### 6.2.2.4. Glucocorticoid induced-atrophy

Glucocorticoid is an endocrine hormone released in several pathological states (sepsis, renal failure, fasting, immobilization, metabolic acidosis, etc.) associated with skeletal muscle atrophy and weakness (192). A recent study showed that the glucocorticoid dexamethasone (Dex) induces skeletal muscle atrophy and stimulates miR-1 expression through glucocorticoid receptor and myostatin (193). These authors proposed a model by which miR-1 inhibits the expression of HSP70 (heat shock protein 70) protein, thereby reducing AKT phosphorylation and enhancing the activation of FOXO3 and atrophy-related proteins. Although FOXO3 was recently shown to be directly down-regulated by miR-155 in glioma cells (194), to our knowledge, no miRNAs have been identified to directly target FOXO3 in skeletal muscle. These results provide evidence that miR-1 is a catabolic miRNA involved in glucocorticoid-induced skeletal muscle atrophy. In addition to the increase in miR-1 levels, Dex reduced miR-27 content in myotubes, a result consistent with the increased expression of its validated target myostatin (195, 196). These findings suggest that myostatin could be a central regulator of the catabolic response through its interaction with miR-1 and miR-27. Furthermore, Dex treatment reduced the level of miR-23 in C2C12 myotubes through a mechanism involving attenuated calcineurin signaling pathway and miR-23 packaging into exosomes (197). Although miR-23a expression was not affected by Dex treatment in skeletal muscle, this miRNA prevented Dex-induced atrophy, most probably by repressing the translation of the ubiquitin-ligases MURF1 and FBXO32 (also called MAFBX or ATROGIN1) (198). These findings provide additional evidence that miR-23 may be a beneficial target to prevent muscle atrophy associated with catabolic states.

### 6.2.3. Disuse-related muscular disorder

To our knowledge, only a few studies have examined the expression of miRNA in skeletal muscle atrophy in response to disuse in animals (133, 199, 200) or humans (201, 202). Allen and colleagues (2009) reported a decreased level of miR-206 expression in the gastrocnemius muscle of mice after a spaceflight, while a trend toward a decrease in miR-133a expression was also note (199). Given that miR-206 is more abundant in slow oxidative than fast glycolytic muscles (128), it was suggested that miR-206 down-regulation in spaceflight muscle may reflect the slow-to-fast shift associated with microgravity exposure. We previously observed that the expression of miR-499 and miR-208b, two myomiRs encoded by Myh7b and Myh7 genes, respectively, were down-regulated after 28 days of hind limb unloading (200). This miRNA dysregulation was associated with the up-regulation of Sox6 (SRY-box containing gene 6), a validated target gene of miR-499 and miR-208b known to repress β-MHC expression in skeletal muscle (37). These findings provide evidence that the dysregulation of these two components of the myomiR network (203), may be involved in the slow-to-fast transition associated with unloading. In addition to the effect on fiber type, miR-499 and miR-208a were shown to repress myostatin expression (45, 204), a repressor of muscle growth that is over-expressed in skeletal muscle following hind limb suspension (205). Among
the four up-regulated miRNAs observed after five days of hind limb immobilization in mice, the increase in miR-696 level was associated with a decrease in the expression of its predicted target gene Pgc-1α (133). These authors demonstrated that miR-696 represses Pgc-1α translation in vitro, suggesting that miR-696 is involved in the impaired oxidative metabolism observed in this model of physical inactivity. The miRNA expression profile performed in human skeletal muscle after 10 days of bed rest revealed the dysregulation of 15 miRNAs, including the down-regulation of miR-206 and miR-23a (201). Although the decreased level of miR-23a was not confirmed by others after seven days of bed rest in human (199), this result provides additional evidence that this miRNA may be involved in the atrophic response associated with this catabolic state (179, 180).

6.3. Aging
Sarcopenia is characterized by a progressive decline in skeletal muscle mass and function that occurs with aging. Recent lines of evidence indicate that miRNA expression is modulated in skeletal muscle during aging. Profiling of miRNA expression in skeletal muscle of mice (206) and rhesus monkeys (207) revealed that several miRNAs involved in the regulation of myogenesis are dysregulated during aging. Compared to young animals, skeletal muscle from both old mice and monkeys showed a decreased expression of miR-181a, a change that could promote the expression of its predicted target Acvr2a (activin receptor IIA), a gene shown to inhibit myogenic cell proliferation through Smad2/3 phosphorylation (208). Therefore, miR-181a down-regulation may impair the proliferation of satellite cells and their ability to repair damaged fibers in aged muscle. A reduction in miR-221 level, a miRNA involved in the regulation of myogenic differentiation (209), was reported in skeletal muscle of aged mice (206), whereas a decline in miR-489, a miRNA highly expressed in quiescent satellite cells (67), was observed in muscle of aged monkeys (207). Drummond and colleagues (2011) observed in skeletal muscle of aged human subjects an increased content of let-7b and let-7e, a finding associated with the down-regulation of several let-7 target genes involved in the regulation of cell cycle (210). Currently, it remains to be examined whether these myogenesis-related miRNAs contribute to the loss of muscle mass in the elderly. Increased levels of the primary miRNAs pri-miR-1-1, pri-miR-1-2, pri-miR-133a-1 and pri-miR-133a-2 were found in old compared to young human subjects, while no changes were observed for the mature miRNAs (129). These authors showed that miR-1 level was reduced in the young, but not old subjects after an acute bout of resistance exercise combined with essential amino acid ingestion (129). Conversely, old human subjects exhibited a reduction in miR-1 expression in response to 12 weeks of resistance training, while this change was not assessed in young individuals (211). The expression of miR-451 was increased in skeletal muscle of old compared to young monkeys (207), and a similar result was reported in "low responders" compared to "high responders" to resistance exercise (32). Additional experiments are required to elucidate whether miR-1, which was shown to inhibit various component of the IGF-I/AKT signaling pathway (130), and miR-451, could be involved in the muscle resistance to anabolic stimuli in the elderly.

6.4. Rhabdomyosarcoma (RMS)
RMS, the most common pediatric soft tissue sarcoma, is derived from skeletal muscle progenitor cells that maintain a proliferative capacity but poorly differentiate (212). As miRNAs play a role in the regulation of both tumorigenesis and myogenesis, intensive efforts have been made to identify miRNAs involved in RMS tumor (213). A dramatic decrease in miR-1 and miR-206 expression was reported in RMS cell line and human RMS specimens, resulting in the up-regulation of the oncogene c-Met, a validated target gene (214). In addition to the down-regulation of miR-1 and miR-206, decreased levels of miR-133a and miR-133b were also observed in RMS tumor samples (88, 215, 216), while low miR-206 expression was shown to correlate with poor
overall survival in RMS patients (216). The fact that the growth and migration of cells derived from a RMS lineage was reduced after transfection of miR-1 and miR-206 suggests that these miRNAs could be potent tumor suppressors in RMS (214).

Several non-myomiRs have also been reported to regulate tumor formation in RMS cell lines. MiR-29, which promotes myogenic differentiation, is repressed by the NFκB/YY1 pathway in RMS cells and primary tumors (88). The inhibition of tumor growth and stimulation of differentiation in RMS cells ectopically over-expressing miR-29 suggests that this miRNA acts as a RMS tumor suppressor. MiR-203 is also down-regulated in both RMS biopsies and cell lines, and its re-expression in RMS cells impaired the cell migration and proliferation, and promoted myogenic differentiation (217). These authors provided evidence that the tumor suppressive effect of miR-203 results from the inhibition of the Notch pathway and the activation of the JAK1 (Janus kinase 1)/STAT1 (signal transducer and activator of transcription 1)/STAT3 pathway. A reduced level of miR-26a was also reported in both RMS cell lines and tumor samples, a result associated with a concomitant up-regulation of Ezh2, a negative regulator of muscle differentiation (218). Recently, Sarver and co-workers (2010) identified miR-183 as a potential oncogenic miRNA, through its ability to blunt the expression of the two tumor suppressors Egr1 (early growth response 1) and Pten (219).

7. CONCLUSIONS

Over the past decade and a half, miRNAs have emerged as another key component of gene regulation underlying the skeletal muscle phenotype. In vitro and in vivo studies have confirmed their important role in myogenesis, however whether they function to maintain muscle throughout adulthood is less clear. Furthermore, during periods of muscle adaptation there are alterations in gene expression, with mounting evidence showing that miRNA accompany these changes, however evidence linking cause-and-effect between gene expression and miRNA levels is still lacking. With this in mind, future research should focus on the extent that miRNAs are involved in maintenance of adult skeletal muscle with the hope of identifying whether the dysregulation of miRNA expression is casual to the progressive loss of muscle mass with disuse or aging. Furthermore, miRNA have been shown to be dysregulated in various myopathies, so both cases represent possibilities where miRNAs may be therapeutic targets that can be modulated. Similarly, miRNAs could act as therapeutics themselves by harnessing their ability to post-transcriptionally regulate gene targets that are dysfunctional resulting in a disease phenotype. Finally, defining the extent to which miRNAs are involved in muscle adaptation to exercise may provide an avenue to develop more effective training programs for those populations in which skeletal muscle adaptation is compromised, such as during aging. Regardless of these challenges, the field of miRNAs in skeletal muscle biology is still in its infancy with many exciting questions still left to be answered.

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9. REFERENCES


11. Y. Lee, M. Kim, J. Han, K. H. Yeom, S. Lee, S. H. Baek and V. N. Kim: MicroRNA genes are transcribed by RNA polymerase II. EMBO J, 23(20), 4051-60 (2004) DOI: 10.1038/sj.emboj.7600385
Function of microRNA in skeletal muscle


33. D.M. Patrick, C.C. Zhang, Y. Tao, H. Yao, X. Qi, R.J. Schwartz, L.J-S. Huang,
Function of microRNA in skeletal muscle


38. E. van Rooij, L. B. Sutherland, X. Qi, J. A. Richardson, J. Hill and E. N. Olson: Control of stress-dependent cardiac growth and gene expression by a microRNA. Science, 316(5824), 575-9 (2007) DOI: 10.1126/science.1139089


46. N. Liu, A. H. Williams, J. M. Maxeiner, S. Bezprozvannaya, J. M. Shelton, J. A. Richardson, R. Bassel-Duby and E. N. Olson: microRNA-206 promotes
DOI: 10.1172/JCI62656

DOI: 10.1126/science.1181046

DOI: 10.1016/j.cell.2007.03.030

DOI: 10.1523/JNEUROSCI.1732-12.2012

DOI: 10.1016/j.molcel.2010.09.027

DOI: 10.1083/jcb.200603008

DOI: 10.1038/ng1725

DOI: 10.1101/gad.1738708

DOI: 10.1172/JCI62667

DOI: 10.1101/cshperspect.a008342

DOI: 10.1016/S0959-437X(00)00215-X

DOI: 10.1016/j.gde.2006.08.008


DOI: 10.1016/j.stem.2012.04.001

60. Z. Yablonka-Reuveni: The skeletal muscle satellite cell: still young and fascinating at 50. J Histochem Cytochem, 59(12), 1041-59 (2011)
DOI: 10.1369/0022155411426780

DOI: 10.1152/physrev.00043.2011
Function of microRNA in skeletal muscle


63. Z. Chen, S. Liang, Y. Zhao and Z. Han: miR-92b regulates Mef2 levels through a negative-feedback circuit during Drosophila muscle development. Development, 139(19), 3543-52 (2012) DOI: 10.1242/dev.082719


68. C. G. Crist, D. Montarras and M. Buckingham: Muscle satellite cells are primed for myogenesis but maintain quiescence with sequestration of Myf5 mRNA targeted by microRNA-31 in mRNP granules. Cell Stem Cell, 11(1), 118-26 (2012) DOI: 10.1016/j.stem.2012.03.011


75. D. Zhang, X. Li, C. Chen, Y. Li, L. Zhao, Y. Jing, W. Liu, X. Wang, Y. Zhang, H. Xia, Y. Chang, X. Gao, J. Yan and H. Ying: Attenuation of p38-mediated miR-1/133 expression facilitates myoblast proliferation during the early stage of
Function of microRNA in skeletal muscle


Function of microRNA in skeletal muscle


130. L. Elia, R. Contu, M. Quintavalle, F. Varrone, C. Chimenti, M. A. Russo, V. Cimino, L. De Marinis, A. Frustaci, D. Catalucci and G. Condorelli: Reciprocal
Function of microRNA in skeletal muscle

regulation of microRNA-1 and insulin-like growth factor-1 signal transduction cascade in cardiac and skeletal muscle in physiological and pathological conditions. Circulation, 120(23), 2377-85 (2009) DOI: 10.1161/CIRCULATIONAHA.109.879429


142. S. Radom-Aizik, F. Zaldivar, Jr., S. Y. Leu, G. R. Adams, S. Oliver and D. M. Cooper: Effects of exercise on microRNA expression in young males peripheral...
DOI: 10.1111/j.1752-8062.2011.00384.x

DOI: 10.1152/japplphysiol.01341.2012

DOI: 10.1113/jphysiol.2011.213363

DOI: 10.1113/jphysiol.2011.213363

DOI: 10.1371/journal.pone.0070823

DOI: 10.1152/japplphysiol.00075.2013


DOI: 10.1371/journal.pone.0057496


DOI: 10.1152/ajpheart.00075.2013

DOI: 10.1371/journal.pone.0087308

DOI: 10.1016/S0140-6736(02)07815-7

DOI: 10.1016/S0140-6736(02)07815-7

DOI: 10.1073/pnas.0708115104

DOI: 10.1038/cdd.2013.62


Function of microRNA in skeletal muscle


181. L. Q. Jiang, N. Franck, B. Egan, R. J. Sjogren, M. Katayama, D.
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Abbreviations: miRNA: microRNA; TGF-β: Transforming growth factor-beta; PAX3: paired-box 3; PAX7: Paired-box 7; MYOD: myogenic differentiation; MYF5: Myogenic factor 5; MRF4: Myogenic regulatory factor 4; Mef2: Myocyte enhancing factor; SRF: serum response factor; HDAC4: Histone deacetylase 4; EZH2: Enhancer of zeste homolog 2; YY1: Ying yang 1; mTOR: mechanical target of rapamycin; IGF: Insulin-like growth factor; PTEN: phosphatase and tensin homolog; CKD: chronic kidney disease; RMS: rhabdomyosarcoma; DMD: Duchenne muscular dystrophy; FSHD: Facioscapulohumeral muscular dystrophy; ALS: Amyotrophic lateral sclerosis; Dex: Dexamethasone, NMJ: neuromuscular junction

Key Words: MyomiR, Exercise, Hypertrophy, Atrophy, Muscular dystrophy, Review

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