MicroRNA in skeletal muscle development, growth, atrophy, and disease

Anja Kovanda,1,2† Tadeja Režen2† and Boris Rogelj1,2∗

MicroRNAs (miRNAs) are short noncoding RNAs that are important global- as well as tissue- and cell-type-specific regulators of gene expression. Muscle-specific miRNAs or myomirs have been shown to control various processes in skeletal muscles, from myogenesis and muscle homeostasis to different responses to environmental stimuli, such as exercise. Importantly, myomirs are also involved in the development of muscle atrophy arising from aging, immobility, prolonged exposure to microgravity, or muscular and neuromuscular disorders. Additionally, muscle atrophy is both induced by and exacerbates many important chronic and infectious diseases. As global yet specific muscle regulators, myomirs are also good candidates for therapeutic use. Understanding the dynamics of myomirs expression and their role in the development of disease is necessary to determine their potential for muscle atrophy prevention. © 2014 John Wiley & Sons, Ltd.

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INTRODUCTION

MicroRNAs (miRNAs) are short noncoding RNA molecules approximately 22 nucleotides in length, which are important regulators of transcriptional and post-transcriptional gene expression in eukaryotes.1–9 Their biogenesis has been extensively studied and will not be covered by this review.10,11 miRNAs can be coded either in protein-coding genes or in non-coding intergenic regions, in either sense or antisense orientation.12 Recently, some miRNAs have also been shown to originate from RNA splicing or endogenous RNAs.13 Most animal miRNAs are complementary to their target mRNA in the ‘seed region’ (nucleotides 2–8)4,5; however, ‘seedless’ binding has also been documented.14,15 Commonly, a single miRNA can often bind up to several hundred mRNAs, while several different miRNAs can bind the same mRNA.5 Computationally predicted as well as some validated targets of miRNAs can be accessed through web-based tools such as Microcosm (http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/) and ncRNA KnowledgeBase (http://www.ncbi.nlm.nih.gov/LinkDB/LinkSet?db=mirror&dbfrom=ncRNA&report=mirna Targets). Myomirs are a large group of miRNAs enriched in cardiac and/or skeletal muscle (Table 1). The term was originally coined for the family of miRNAs (miR-208/miR-499) found in introns of myosin genes16; however, the definition has expanded to include all muscle-enriched miRNAs. So far, myomirs have been shown to govern several key processes, from myogenesis to fiber shift, muscle growth, and atrophy.17–21 Additionally, more than 190 miRNAs have been shown to be dysregulated in many pathological conditions18,21 and some have shown potential as targets of therapeutic intervention.10,22–25 Some myomirs can also be found in other tissues, and can therefore be considered as either muscle exclusive or nonexclusive depending on their additional locations and functions.21,26 Although many of the muscle-exclusive cardiac and skeletal muscle myomirs overlap, this review will focus in detail on the functions of miRNAs in skeletal muscle.
### TABLE 1 | Myomirs Tissue Specificity and Process Involvement

<table>
<thead>
<tr>
<th>Process</th>
<th>Muscle-Exclusive and Enriched Myomirs</th>
<th>Ubiquitously Expressed Myomirs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myogenesis</td>
<td>miR-1, miR-27, miR-27b, miR-133a/b, miR-206, miR-208a/b, miR-499a/b, miR-486, miR-367, miR-489</td>
<td>miR-245, miR-26a, miR-297, miR-125b, miR-181, miR-214, miR-221, miR-222, miR-322, miR-503, miR-682, miR-23b, miR-28, miR-31, miR-98, miR-103, miR-107, miR-193a, miR-210, miR-324, miR-324, miR-331, miR-374, miR-432, miR-502, miR-451, miR-452, miR-565, miR-594, miR-660, miR-659</td>
</tr>
<tr>
<td>Homeostasis/adult myogenesis</td>
<td>miR-1, miR-206, miR-499a, miR-23a</td>
<td>miR-23a</td>
</tr>
<tr>
<td>Fiber shift</td>
<td>miR-499</td>
<td>miR-23a</td>
</tr>
<tr>
<td>Physical activity</td>
<td>miR-1, miR-23a, miR-133a/b, miR-206</td>
<td>miR-107, miR-181, miR-486</td>
</tr>
<tr>
<td>Aging</td>
<td>miR-27a, miR-27b, miR-133a, miR-133b</td>
<td>let-7a/b/e/f, miR-25, miR-98, miR-195, miR-1268, miR-22, miR-24, miR-30d, miR-223, miR-278</td>
</tr>
<tr>
<td>Atrophy</td>
<td>miR-1, miR-133a, miR-206, miR-23a, miR-208b, miR-499a, and miR-23b</td>
<td>let-7a/b/d/e/g, miR-30b, miR-98, miR-101, miR-145, miR-148, miR-199, miR-29, miR-107, miR-696</td>
</tr>
<tr>
<td>Regeneration</td>
<td>miR-206, miR-23a</td>
<td>miR-222</td>
</tr>
<tr>
<td>Neuromuscular regeneration</td>
<td>miR-1, miR-133, miR-206, miR-206</td>
<td>miR-181, miR-682</td>
</tr>
</tbody>
</table>

miRNA IN MYOGENESIS

Skeletal muscles are formed during embryonic development and require highly coordinated assembly. First, fibroblast growth factor (FGF) causes proliferation of myoblasts or muscle progenitor cells. The myoblasts then align, exit the cell cycle, and start differentiation, finally fusing to form multinucleated fibers called myotubes. This differentiation process is activated by myogenic regulatory factors (MRFs) of the MyoD family and myocyte enhancer factor-2 (MEF2) family of transcription factors, which enable muscle-specific gene transcription.

miRNA-mediated regulation is required for successful myogenesis. In cardiac myocytes the deletion of DGCR8, an essential part of miRNA biogenesis machinery, results in heart failure. Similarly, inactivation of DICER, which is involved in biogenesis of several small RNAs including miRNAs, during embryonic myogenesis in mice results in notably decreased skeletal muscles and perinatal lethality.

In myogenesis, proliferation and differentiation are mutually exclusive, and miRNAs are importantly involved in balancing these two processes (Figure 1). Several essential myomirs involved in myogenesis (Table 1), as well as their regulatory feedback loops, have been reviewed in great detail.20

Starting with miRNAs exclusively expressed in cardiac and skeletal muscles, the myomirs that show the most dramatic increase during myoblast differentiation are miR-1, miR-206, miR-133a, and miR-133b. They are involved in skeletal myogenesis, and control satellite and myoblast cell proliferation and differentiation. The four myomirs are expressed as miR-1/miR-133a and miR-206/miR-133b bicistronic transcripts from three chromosomal loci under the control of myogenic transcription factors: serum response factor (SRF), MyoD, and MEF2. miR-206 is expressed exclusively in skeletal muscles, where its transcription is induced by MyoD and myogenin.

Although they originate from the same transcript, miR-1 and miR-133 have specific roles in muscle proliferation and differentiation. miR-133 enhances proliferation by repressing SRF, whereas miR-1 guides the myoblasts toward differentiation by repressing histone deacetylase 4 (HDAC4), an inhibitor of muscle differentiation. Recently, an additional role has been identified for miR-133 in mice where the repression of this myomir family enables the
satellite cells to differentiate into brown adipose tissue rather than myocytes. However, such role of miR-133 in humans has not yet been shown.

Similarly to miR-1, miR-206 also promotes muscle differentiation while repressing proliferation of myocyte progenitors.73,77

Another group of muscle-exclusive myomirs are miR-208a, miR-208b, and miR-499, which control muscle fiber types, and are transcribed from introns of host myosin genes MYH6, MYH7, and MYH7b, respectively.16,83 miR-208b and miR-499 are found in both cardiac and skeletal muscle, with miR-499 being implicated in skeletal muscle maintenance,48 whereas miR-208a is enriched in cardiac muscle.84

Myogenic, muscle-enriched miR-486, encoded in the gene for Ankyrin 1 (ANK1), is also induced during myoblast differentiation.85 Its expression is activated by SRF, MyoD, and myocardin-related transcription factor A (MRTF-A).

Myogenesis additionally involves several other muscle nonexclusive miRNAs, such as miR-2435; miR-26a36; miR-2722,86; miR-2955,37; miR-18140; miR-21441,42 and miR-322/424/50344 that become upregulated during myoblast differentiation, and miR-23a49; miR-125b19 and miR-221/22243 that become downregulated during myoblast differentiation. Recently, additional 20 miRNAs were shown to be either upregulated (miR-23b, miR-28, miR-98, miR-103, miR-107, miR-193a, miR-210, miR-324-5p, miR-324-3p, miR-331, miR-374, miR-432, miR-502, and miR-660) or downregulated (miR-31, miR-451, miR-452, miR-565, miR-594, and miR-659) upon induced differentiation in cultured human myoblasts.46

Finally, miR-682 was shown to be upregulated in mouse proliferating myogenic progenitor cells; however, its significance in humans is unclear owing to the lack of homologous miRNA.

**miRNAs in Adult Muscle Homeostasis, Fiber Shift, and Exercise**

While some of the ubiquitously expressed miRNAs seem to be required mostly during myogenesis, several muscle-exclusive and muscle-enriched myomirs expressed during myogenesis remain present in adult muscles and act in muscle homeostasis, fiber shift, growth, and regeneration.47,48,61,72,87

**Structural Features of Skeletal Muscle Tissue**

The high number of myomirs found in skeletal muscle reflects its nature as a complex and highly ordered tissue consisting of several cell types. The main cell types represented are the muscle cells or myocytes organized into bundles or fibers, which are held together by connective tissue. In addition, skeletal muscles have a good blood supply with many capillaries and are well enervated, being under control of the somatic nervous system. During atrophy/disease, skeletal muscles can be infiltrated by immune cells and adipose tissue.
TABLE 2 | Fiber Type Classification and Characteristics

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>Type I</th>
<th>Type IIa</th>
<th>Type IIx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synonyms</td>
<td>Slow-oxidative</td>
<td>Fast-oxidative</td>
<td>Fast-glycolytic</td>
</tr>
<tr>
<td>Myosin</td>
<td>MYH7</td>
<td>MYH2</td>
<td>MYH4</td>
</tr>
<tr>
<td>Mitochondria content</td>
<td>Very high</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Capillaries content</td>
<td>High</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Oxidative capacity</td>
<td>High</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Glycolytic capacity</td>
<td>Low</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Contraction velocity</td>
<td>Slow</td>
<td>Fast</td>
<td>Fast</td>
</tr>
<tr>
<td>Fatigue resistance</td>
<td>High</td>
<td>Medium</td>
<td>Very low</td>
</tr>
</tbody>
</table>
however, the authors show that in humans the determinants of muscle endurance correlate only with the expression of estrogen-related receptor $\gamma$ (ERR$\gamma$) and miR-499, suggesting these mechanisms to be species specific.\(^{50}\)

miR-499 has also been implicated in muscle maintenance and increased muscle endurance.\(^{16,48,94}\) Interestingly, in addition to skeletal and cardiac muscles, MYH7b (the gene where miR-499 is encoded) has been found to be expressed in a subset of neurons,\(^{95}\) an association that merits further investigation.

miR-486 is encoded in the intron of ANK1 and is enriched in muscle after exercise in mice.\(^{54}\) It is suggested to target phosphatase and tensing homolog (PTEN) and FOXOa1 involved in the phosphoinositide-3-kinase/Akt signaling, an important insulin signaling pathway.\(^{96}\) In a recent study examining changes in circulating myomirs (miR-1, miR-133a, miR-133b, miR-206, miR-206b, miR-486, and miR-499), only miR-486 was found to be significantly reduced in serum owing to acute or chronic exercise in healthy young males.\(^{54}\) The authors suggest that miR-486 is reduced in serum during exercise, owing to its uptake by skeletal muscle, where it aids glucose uptake by suppression of PTEN and activation of insulin signaling.\(^{54}\)

In addition to the mentioned muscle-enriched myomirs, several other miRNAs involved in pathways regulating mitochondrial biogenesis, glucose and fatty acid metabolism, and skeletal muscle remodeling have been shown to change owing to acute exercise in animal studies. miR-1, miR-107, and miR-181 were found to be increased, whereas miR-23a was down-regulated in skeletal muscle of mice following forced acute endurance exercise.\(^{51}\) miR-1 and miR-181 both have a recognized role in myogenesis\(^{20,40}\) and may be increased as a means to sustain muscle mass. miR-107, encoded in the intron of pantothenate kinase 1 (PANK1) gene, is a predicted regulator of pyruvate dehydrogenase kinase 4 (PDK4).\(^{97}\) miR-23a is a predicted regulator of PPARGC1 (PGC1$\alpha$), the expression of which increases mitochondrial content and oxidative capacity and drives transition from the fast type II to the slow type I muscle fibers.\(^{98}\) Additionally, miR-23a was recently shown to repress expression of fast myosin heavy-chain isoforms in mice,\(^ {49}\) and MAFbx/atrogen-1 and muscle RING-finger 1 (MuRF1), two muscle-specific ubiquitin ligases involved in atrophy-associated protein degradation.\(^ {62}\)

Of note is that animal experiments used to examine the relationship between the miRNA expression and exercise, such as forced acute endurance experiments, have several intrinsic problems that may substantially affect measured miRNA and mRNA levels. Both are likely affected by the substantial differences in skeletal muscle size, the rate of muscle growth, and the lifetime duration between rodents and humans. miRNA expression is also likely affected by the general unfitness of laboratory animals, owing to the lack of movement\(^ {99}\), and the presence of acute
stress factors\textsuperscript{100}, such as functional overload and electric shocks in forced endurance exercise. Despite high evolutionary conservation of miRNAs, several recent studies have shown that there are important species-specific molecular differences.\textsuperscript{50,101,102} Indeed, homologs of some mouse miRNAs involved in important aspects of skeletal muscle regulation have yet to be found in humans, but are known to affect behavior of molecular targets with human equivalents.\textsuperscript{45,91} And similarly, some important human myomirs such as miR-133 may be redundant in mouse models of disease.\textsuperscript{103} Results of animal studies should be treated with these issues in mind.

**MYOMIR INVOLVEMENT IN ATROPHY**

Atrophy of skeletal muscles can result from either primary or secondary muscle disorders or from inactivity and/or aging in otherwise healthy individuals. In order to distinguish between miRNA changes in ‘normal’ and disease-induced muscle atrophy, it is essential to compare both processes.

**Atrophy in Healthy Individuals**

In healthy individuals, skeletal muscle atrophy results from prolonged immobility, aging, caloric restriction, physical inactivity, or special microgravity conditions, such as space flight, and is characterized by reduced muscle strength, lower synthesis and higher protein degradation rate, protein carbonylation, shift in muscle fiber type from slow type I to fast type II, increased oxidative stress, development of insulin resistance, and intramuscular fat deposits.\textsuperscript{104–111}

Age was shown to influence myomir expression in a study comparing skeletal muscle biopsies of young and older healthy men.\textsuperscript{56} In older men, let-7\textsubscript{a/b/e/f} and miR-25, miR-98, miR-195, and miR-1268 were upregulated, whereas miR-22, miR-24, miR-27a, miR-27b, miR-30d, miR-133a, miR-133b, miR-223, and miR-278 were downregulated compared with young subjects.\textsuperscript{56} Interestingly, there was no difference in the expression of miR-206 between both groups.

Bed rest studies performed on healthy human individuals, as well as mouse and rat atrophy models, show downregulation of myomirs, which allows derepression of pathways such as insulin signaling, TNF, transforming growth factor β (TGF-β), Smad2, and MAPK leading to development of atrophy, insulin resistance, and metabolism and fiber type shift.\textsuperscript{48,55,57,58,112} Experiments examining atrophy-associated myomir expression changes

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**TABLE 3 | Expression of Atrophy-Associated Myomirs in Healthy Models**

<table>
<thead>
<tr>
<th>Myomir</th>
<th>Healthy Humans</th>
<th>Animal Models</th>
</tr>
</thead>
<tbody>
<tr>
<td>Let-7 a/b/c/d/e/g</td>
<td>↓10d bed rest/male\textsuperscript{57}</td>
<td>~11d (mice/space flight)\textsuperscript{112}</td>
</tr>
<tr>
<td>miR-1</td>
<td>↓7d bed rest/male\textsuperscript{58}</td>
<td>~11d (mice/space flight)\textsuperscript{112}</td>
</tr>
<tr>
<td>miR-133a</td>
<td>↓7d bed rest/male\textsuperscript{58}</td>
<td>~11d (mice/space flight)\textsuperscript{112}</td>
</tr>
<tr>
<td>miR-206</td>
<td>~7d bed rest/male\textsuperscript{58}</td>
<td>↓11d (mice/space flight)\textsuperscript{112}</td>
</tr>
<tr>
<td>miR-208b</td>
<td>↓2–7d (rat/HLS)\textsuperscript{48}</td>
<td></td>
</tr>
<tr>
<td>miR-499</td>
<td>↓2–7d (rat/HLS)\textsuperscript{48}</td>
<td></td>
</tr>
<tr>
<td>miR-23a</td>
<td>~7d bed rest/male\textsuperscript{58}</td>
<td>↓2–7d (rat/HLS)\textsuperscript{48}</td>
</tr>
<tr>
<td>miR-23b</td>
<td>↓10d bed rest/male\textsuperscript{57}</td>
<td></td>
</tr>
<tr>
<td>miR-29</td>
<td>↓7d bed rest/male\textsuperscript{58}</td>
<td>↓2–7d (rat/HLS)\textsuperscript{48}</td>
</tr>
<tr>
<td>miR-30b</td>
<td>↑10d bed rest/male\textsuperscript{57}</td>
<td></td>
</tr>
<tr>
<td>miR-98</td>
<td>↓10d bed rest/male\textsuperscript{57}</td>
<td></td>
</tr>
<tr>
<td>miR-101</td>
<td>↑10d bed rest/male\textsuperscript{57}</td>
<td></td>
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<tr>
<td>miR-107</td>
<td>↓2–7d (rat/HLS)\textsuperscript{48}</td>
<td></td>
</tr>
<tr>
<td>miR-145</td>
<td>↓10d bed rest/male\textsuperscript{57}</td>
<td></td>
</tr>
<tr>
<td>miR-148</td>
<td>↓10d bed rest/male\textsuperscript{57}</td>
<td></td>
</tr>
<tr>
<td>miR-199</td>
<td>↓10d bed rest/male\textsuperscript{57}</td>
<td></td>
</tr>
<tr>
<td>miR-696</td>
<td>↑5d (mice/HLS)\textsuperscript{55}</td>
<td></td>
</tr>
</tbody>
</table>

↓, downregulated; ↑, upregulated; ∼, unchanged; HLS, hindlimb suspension; d, duration in days.
in healthy individuals and/or animals are listed in Table 3.

As illustrated by these studies, comparison of results is often difficult. How the selection of identification method can affect results has been reviewed previously.\textsuperscript{113} In case of atrophy, interpretation of results is additionally complicated by the nature of the process. Skeletal muscle tissue undergoes global changes during atrophy, and even muscle biopsy samples taken from the same individual before and after atrophy substantially differ in proportion of fiber types, as well as overall muscle, vascular (capillary), and adipose cell content. Therefore, direct comparison of miRNAs in tissue biopsies is difficult and ideally, the proportion of different cell types in the biopsy material should be taken into account.

In skeletal muscle tissue, myomirs both regulate and are regulated by the processes taking place during atrophy. Furthermore, many myomirs are regulated by positive and negative feedback loops and determining cause and effect of miRNA expression in skeletal muscle atrophy remains challenging. Several of the ‘healthy’ atrophy-associated myomirs, such as miR-1, miR-133, miR-30b, and miR-206 are repressed by insulin in human skeletal muscle tissue,\textsuperscript{114} while at the same time are involved in insulin signaling pathways. Such myomir regulatory loops (Figure 3) are likely to be affected in diseased as opposed to healthy individuals.

\textbf{Disease-Associated Atrophy}

Changes in expression of myomirs have been shown both in primary muscular and neuromuscular atrophies, and secondary muscular disorders, such as cancer, diabetes, chronic kidney disease, chronic obstructive pulmonary disease, and heart failure.\textsuperscript{25,53,59,60}

A study comparing miRNA expression in muscle tissue in Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), limb-girdle muscular dystrophy (LGMD) type 2A and type 2B, Miyoshi myopathy (MM), facioscapulohumeral muscular dystrophy (FSHD), nemaline myopathy (NM), inclusion body myositis (IBM), polymyositis (PM), and dermatomyositis (DM) found 185 miRNAs to be differently expressed in these diseases as opposed to healthy controls.\textsuperscript{59} Of note, the study did not use age-matched healthy muscle tissue controls, but rather a ‘reference tissue’ created from several tissue datasets, which somewhat limits the findings. Of the 185 differentially expressed miRNAs, only miR-146, miR-155, miR-214, miR-221, and miR-222 were dysregulated in all 10 primary muscular disorders, reflecting their diverse etiology.\textsuperscript{59}
### TABLE 4 | Expression of Atrophy-Associated Myomirs in Primary and Secondary Muscular Diseases

<table>
<thead>
<tr>
<th>Experimental Model</th>
<th>Human</th>
<th>Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary muscular and neuromuscular disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duchenne muscular dystrophy (DMD)</td>
<td>↑miR-1, ↑miR-29c, ↑miR-135a, ↑miR-124a, ↑miR-31, ↑miR-34c, ↑miR-206, ↑miR-222, ↑miR-223, ↑miR-335, ↑miR-449, ↑miR-494↑miR-486↑miR-31, ↑miR-133, ↑miR-206, ↑miR-199a</td>
<td>CXMDJ dogs↑miR-1, ↑miR-133, ↑miR-206 MDX mice↑miR-29c MDX mice↑miR-124a, ↑miR-135a, ↑miR-516-3p, ↑miR-31, ↑miR-34c, ↑miR-206, ↑miR-335, ↑miR-449, ↑miR-494, ↑miR-222, ↑miR-223 MDX mice↑miR-29 MDX mice↑miR-31 MDX mice↑miR-1, ↑miR-133, ↑miR-206 MDX mice↑miR-222 MDX mice↑miR-223 human myoblast cell culture↑miR-1, ↑miR-133, ↑miR-206, ↑miR-455 MDX mice↑miR-31 MDX mice↑miR-1, ↑miR-133, ↑miR-206 MDX mice↑miR-222 MDX mice↑miR-223 MDX mice↑miR-223 MDX mice↑miR-31 MDX mice↑miR-9↑miR-486</td>
</tr>
<tr>
<td>Amyotrophic lateral sclerosis (ALS)</td>
<td>↑miR-23a, ↑miR-29b, ↑miR-206, ↑miR-455</td>
<td>G93A-SOD1 mice↑miR-206, ↑miR-133b</td>
</tr>
<tr>
<td>Spinal muscular atrophy (SMA)</td>
<td>↑miR-206</td>
<td>MNDicer-mut mice↑miR-9</td>
</tr>
<tr>
<td>Myotonic dystrophy type 1 (MD1)</td>
<td>↑miR-206</td>
<td></td>
</tr>
<tr>
<td><strong>Secondary muscular disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease (COPD)</td>
<td>↑miR-206</td>
<td>CKD mice↑miR-23a, miR-29a, miR-29b CKD mice↑miR-486</td>
</tr>
<tr>
<td>Chronic kidney disease (CKD)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Additional evidence of myomirs shown to be dysregulated in primary and secondary muscle diseases is listed in Table 4.

**Duchenne Muscular Dystrophy and Becker Muscular Dystrophy**

DMD and BMD are progressive X-linked primary muscular disorders resulting from either full or partial inactivation of the dystrophin gene. In DMD a mutation of dystrophin gene results in a nonfunctional protein and severe degeneration of skeletal and diaphragm muscles followed by respiratory failure. In BMD the dystrophin protein remains partially functioning and the symptoms are consequently less severe. Apart from the Eisenberg study, no statistically significant difference was observed in the expression of myomirs in BMD patients compared to healthy controls. This could either reflect lesser severity of the disease compared with DMD or the consequent lower research coverage of this disorder.

In contrast, myomirs miR-1, miR-29c, and miR-135a were found to be downregulated, whereas miR-31, miR-34c, miR-124a, miR-206, miR-222, miR-223, miR-335, miR-449, and miR-494 were found to be upregulated in DMD patients both compared to controls and compared to BMD patients. An increase in miR-31 was also confirmed in another recent study, where it was suggested that miR-31 regulates dystrophin expression and, therefore, is a potential therapeutic target. Interestingly, a study comparing myomir expression in the serum of DMD patients showed miR-1, miR-133, and miR-206 to be upregulated compared to controls. Myogenic miR-486 is also downregulated in DMD patients, while miR-199a was upregulated in DMD human myoblast cell culture.

**Amyotrophic Lateral Sclerosis**

ALS is a neuromuscular disorder where complex protein/DNA/RNA interplay at the molecular level and cell/tissue interplay at the system level result in loss of motor neurons, denervation, and progressive...
skeletal muscle atrophy. Mutations, mislocalization, and aggregation of two proteins, TDP-43 and FUS, are principally implicated in the disease. The key ALS proteins TPD-43 and FUS, which may in some cases be mutated in ALS, are predominantly nuclear, bind DNA and RNA, and are involved in regulating transcription, splicing, and RNA transport. Furthermore, both are components of the Drosha complex and are involved in enhancing miRNA biogenesis. Their silencing affects neuronal expression of several microRNAs, among which mir-30b, mir-125b, mir-128, mir-145, mir-181, mir-199a, and mir-335 are associated with myogenesis, atrophy, atrophy protection, inactivity, and DMD (Tables 1, 3, and 4). How knockdown of TDP-43 or FUS in skeletal muscle specifically contributes to the development of ALS remains to be determined.

Several myomirs involved in reinnervation have been identified in denervation models. A study done in a rat denervation model showed the expression of atrophy-associated myomirs mir-1, mir-133a, and mir-206 to be time dependent. Following denervation, mir-1 and mir-133a were downregulated 1 month postdenervation, but both myomirs increased 4 months postdenervation and reinnervation. Level of mir-206 was persistently increased 1–4 months after reinnervation, but not following denervation. Of importance, local injection of a cocktail of double-stranded miRNAs containing mir-206, mir-1, and mir-133 has been shown to accelerate muscle regeneration in a rat skeletal muscle injury model.

In a G93A-SOD-1 mouse ALS model, mir-206 and mir-133b were shown to be increased upon disease onset. In the same study, muscle reinnervation was delayed in mir-206 knockout (KO) mice after denervation compared with controls, and, at the same time, loss of mir-206 resulted in an increase in HDAC4, which is also a regulator of neuro muscular gene expression. G93A-SOD1 mir-206 KO mice showed faster disease progression compared with G93A-SOD1 littermates, despite similar disease onset. In contrast, HDAC4 KO mice exhibited enhanced reinnervation. On the basis of this evidence the authors suggest reinnervation to be mir-206 dependent and the observed increase of mir-206 in ALS and ALS mouse model to result from reinnervation attempts. As HDAC4 is also controlled by TGF-β through mir-29, the observed increase in mir-29 in ALS patients may act through a similar mechanism. Supporting this hypothesis, recently, mir-23a, mir-29b, mir-206, and mir-455 have been shown to be increased in skeletal muscle tissue of ALS patients compared with controls. In addition to myomirs, miR-155, increased in spinal chord of ALS patients, has also been suggested as a therapeutic target.

miRNAs in Other Disorders Causing Primary and Secondary Muscle Atrophy
Apart from DMD and ALS, myomir involvement in other primary muscular and neuromuscular disorders has not been studied extensively, but some indications exist of their importance.

Myomir involvement in inclusion body myositis (IBM), an inflammatory muscular disorder has so far been examined only by Eisenberg et al. and recently nitric oxide synthase (NOS) and TWEAK have been shown to be upregulated in some DM1 patients, whereas other miRNAs with predicted target sites in DMPK gene, such as mir-103 and mir-107, were not differently expressed in DM1 patients compared with controls.

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miRNA with their targets, as each miRNA may have several targets and each target may be regulated by several different miRNAs involved in diverse pathways. To complicate matters further, many myomirs, such as miR-23a, are not expressed exclusively in skeletal muscle and have different functions in various other tissues. Additionally, atrophy and regeneration are closely linked via several pathways (Figure 3), many of which have yet to be validated in humans.

Starting from the healthy human subjects, myogenic miRNAs such as miR-1, miR-133, and miR-206 were shown to play a role in human skeletal muscle regeneration in several studies. Interestingly, exercise, as one of the first approaches, was shown to ameliorate secondary muscle atrophy due to chronic kidney disease in mice. Up to date, pharmacological inhibition of myostatin has also been successfully attempted in treating mice with chronic kidney disease and similar experiments leading to human therapy are currently under way. However, given the diverse etiology of many atrophies, additional approaches are needed and several recent studies indicate that myomir therapy could prove useful for the treatment of neuromuscular disorders. These experiments are listed in Table 5.

Several strategies to administer miRNAs or affect their targets have been adopted, but so far been tested only in animal and cell-culture models, where the results are encouraging.

Unfortunately, many of the non-myomir therapeutic intervention studies done on the mdx and G93A-SOD1 mice models failed to translate to useful therapeutic intervention in humans. Given the gravity of muscular dystrophies and availability of control tissue from healthy participants of bed rest studies, carefully designed myomir expression modification experiments on humans with disease should be considered in order to hasten therapy development (Box 1).

**BOX 1**

**MYOMIRS IN HIBERNATION AND HYPERTROPHY**

Myomirs have recently been shown to be involved in mechanisms preventing muscle atrophy during prolonged muscle inactivity in diverse groups of hibernating animals, such as bats and ground squirrels, suggesting a conserved evolutionary mechanism. In torpid bats, miR-1a-1, miR-29b, miR-181b, miR-15a, miR-20a, miR-206, and miR-128-1 were shown to be upregulated, whereas miR-21 was substantially downregulated. Myomirs can, in special cases, also cause hypertrophy, which could serve as an interesting approach for the treatment of atrophy. In Texel sheep, a ‘double muscle’ breed, a mutation in the 3′UTR of myostatin enables the binding of miR-1 and miR-206 and its consequent downregulation and development of muscle hypertrophy.
CONCLUSION

Myomirs are important regulators of molecular processes in muscle tissue and changes in their expression have been shown to guide muscle toward atrophy or regeneration. As such regulators, myomirs show promising therapeutic potential for the treatment of primary and secondary muscle diseases. Improved understanding of constitutive roles of myomirs in skeletal muscle homeostasis as well as their involvement in neuromuscular and muscular diseases will offer new options for slowing down or reversing muscle atrophy.

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REFERENCES

Advanced Review

wires.wiley.com/rna


44. Sarkar S, Dey BK, Dutta A. MiR-322/424 and -503 are induced during muscle differentiation and promote cell cycle quiescence and differentiation by
Functions of miRNA in skeletal muscle


64. Williams AH, Valdez G, Moresi V, Qi X, Macnally J, Elliott JL, Basell-Duby R, Sanes JR, Olson EN. MicroRNA-206 delays ALS progression and promotes regeneration of neuromuscular synapses in


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