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MicroRNA in myogenesis and muscle atrophy

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Abstract

Purpose of review—To understand the impact of microRNA on myogenesis and muscle wasting in order to provide valuable information for clinical investigation.

Recent findings—Muscle wasting increases the risk of morbidity/mortality in primary muscle diseases, secondary muscle disorders and elderly population. Muscle mass is controlled by several different signalling pathways. Insulin-like growth factor/PI3K/Akt is a positive signalling pathway, as it increases muscle mass by increasing protein synthesis and decreasing protein degradation. This pathway is directly and/or indirectly downregulated by miR-1, miR-133, miR-206 or miR-125b, and upregulated by miR-23a or miR-486. Myostatin and the transforming growth factor- β signalling pathway are negative regulators that cause muscle wasting. An increase of miR-27 reduces myostatin and increases muscle cell proliferation. Muscle regeneration capacity also plays a significant role in the regulation of muscle mass. This review comprehensively describes the effect of microRNA on myoblasts proliferation and differentiation, and summarizes the varied influences of microRNA on different muscle atrophy.

Summary—Growing evidence indicates that microRNAs significantly impact muscle growth, regeneration and metabolism. MicroRNAs have a great potential to become diagnostic and/or prognostic markers, therapeutic agents and therapeutic targets.

Keywords

hypertrophy/atrophy; insulin-like growth factor; muscle regeneration; muscle wasting; proliferation and differentiation

INTRODUCTION

MicroRNAs (miRNAs) are evolutionarily conserved, small (17–22 nucleotides) noncoding RNAs. The first miRNA (lin-4) was discovered in *Caenorhabditis elegans* by Victor Ambros, Rosalind Lee and Rhonda Feinbaum in 1993. A second miRNA (Let-7) was characterized in 2000, and shortly thereafter, many miRNAs were identified in many species, indicating the existence of a wider phenomenon [1]. Since then, miRNAs have been the subject of intensive research. More than 21 000 miRNAs have been identified, including more than 1900 in the human genome, that target mammalian mRNA [1]. In general, miRNAs negatively regulate gene expression by post-transcription mechanisms.

miRNAs are involved in a variety of biological processes and diverse pathologic conditions. They influence gene expression in a sequence-specific manner in the following way: specific miRNAs bind to target sequences in the 3'-untranslated region (3'-UTR) of a

complementary mRNA. In plants, the complement is exact, but in mammals, the match can be slightly less than perfect. This binding results in either degradation of the targeted mRNA or inhibition of translation of the targeted mRNA to its corresponding protein. Therefore, the consequence of an increase in a specific miRNA is a decrease in protein product. In many cases, this consequence (miRNA inhibits mRNA downregulating protein production) is not a one-to-one relationship between a specific miRNA and protein because several miRNAs can simultaneously regulate the expression of one protein. Moreover, an individual miRNA can influence the expression of a number of different proteins.

Similar to mRNA, miRNAs are transcribed from genomic DNA. The expression of miRNAs is regulated by their promoter regions. An individual miRNA can be transcriptionally regulated by a number of different transcription factors. The miRNAs are initially transcribed from nuclear DNA producing a primary miRNA (pri-miR). Still in the nucleus, the pri-miR is enzymatically cleaved yielding stem-loop structures of about 70–120 nucleotides, named precursor miRNA (premiR). The premiR is transported to the cytosol where it is further cleaved to produce a mature miRNA that is relatively stable (long half-life). Recall that miRNAs are regulated either by degradation or transcription. An increase in mature miRNA that parallels increased pri-miR and premiR could suggest that this miRNA is regulated at transcription (promoter) levels. Lack of consistency between levels of pri-miR/premiR and mature miRNA would indicate that changes in the latter are due to degradation of this miRNA.

Recently, new findings have added to our knowledge on miRNA biogenesis and regulation. Instead of genomic DNA, some premiRNAs originate from RNA splicing. miRNAs also can be produced from various endogenous RNAs, such as small nucleolar RNAs (snoRNAs), transfer RNAs (tRNAs) and intronic noncoding RNAs [2]. In addition, some transcription factors bind to pri-miRNA rather than a miRNA promoter and affect miRNA processing [1]. Recently, a new regulatory system has been identified in which RNAs can crosstalk with each other by competing for shared miRNAs. Such competing endogenous RNAs (ceRNAs) regulate the distribution of miRNA molecules on their targets (separate miRNA from their targets) and thereby impose an additional level of post-transcriptional regulation. For example, a muscle-specific, long noncoding ceRNA, linc-MD1, regulates muscle differentiation in mouse and human myoblasts. Linc-MD1 influences miR-133 and miR-135 to regulate the expression of Mastermind-like protein 1 and myocyte enhancer factor-2C (MEF2C), respectively. The latter are transcription factors that activate muscle-specific gene expression [3]. Additional detailed information on miRNAs' production and function can be found from a recent review by Goljanek-Whysall *et al.* [1].

MUSCLE-SPECIFIC MICRORNA AND MYOGENESIS

Many miRNAs are expressed in a tissue-specific manner. Accordingly, miRNAs are divided into two categories: miRNAs that are specifically expressed in muscle and not in other tissues (myomiRs) and miRNAs expressed exclusively in nonmuscle tissue or broadly expressed across many cell types. Both categories have significant impacts on muscle proliferation and differentiation. MyomiRs typically control myogenic precursor fate and muscle tissue homeostasis. miR-1, miR-133 and miR-206 fall into this category. miR-1 and miR-133 are expressed in both cardiac and skeletal muscle, and miR-206 is only found in skeletal muscle [4]. Modulation of these miRNAs during myogenesis has been widely studied and reviewed [1,4,5,6,7,8]. In brief, muscle gene expression is regulated by serum response factor (SRF), myocyte enhancer factor 2 (MEF2) and myogenic regulatory factors [also named basic helix-loop helix (bHLH) transcription factors], including myoD, Myf5, MRF4 and myogenin [6]. In skeletal muscle, SRF and MEF2 cooperate with myoD and myogenin to transcriptionally activate the expression of three pairs of muscle-specific

miRNA: miR-1-1 and miR-133a-2 (clustered on mouse chromosome 2 and human chromosome 20), miR-1-2 and miR-133a-1 (clustered on mouse chromosome 18 and human chromosome 18) and miR-206 and miR-133b (clustered on mouse chromosome 1 and human chromosomes 6) [1]. Even if two miRNAs are clustered in the same chromosome, their transcription may be independently controlled [9]. miR-133 increases myoblast proliferation and decreases differentiation; miR-1 and miR-206 decrease myoblast proliferation and increases differentiation. A recent study provides the new evidence that miR-1/miR-206 plays a major role in myoblast differentiation by regulation of multiple target genes, include nine new target proteins. Inhibition of endogenous miR-1 and miR-206 blocks the downregulation of most targets in differentiating cells, such as Notch 3 [10], thus indicating that miRNA activity and target interaction is required for muscle differentiation [11]. Recently, Kang *et al.* [12] used a new technology – dual optical miRNA imaging – to confirm that miR-1 is increased during C2C12 cell differentiation. This technology uses novel imaging processes to provide information about miRNA biogenesis that could be useful during a variety of biologic processes.

When exogenously supplied, myomiRs can act in concert to accelerate muscle regeneration. Local combined application of miR-1, miR-133 and miR-206 induced myoD1, Pax7 and myogenin causing myoblast differentiation in a rat injury model [13]. One study demonstrates that miR-1 and miR-206 directly target (block) Pax 3. Down-regulation of Pax3 is essential to ignite the myogenic programme. Consistent with this, inhibition of miR-1 and miR-206 leads to delayed myogenesis [14]. In another study on muscle remodelling, investigators found that the upregulation of miR-206 directly repressed the expression of high-mobility group protein B3 (Hmgb3), thereby increasing regeneration of single muscle fibres. This suggests that miR-206 could be a viable therapeutic target [15].

Nonmuscle-specific microRNAs and myogenesis

Several nonmuscle-specific miRNAs have also been implicated in myogenesis. Myogenesis includes quiescent satellite cell activation, proliferation, differentiation and fusion. In adult muscle, the myogenic determination gene *Myf5* plays an important role to determine whether satellite cells enter a proliferation/differentiation programme or remain quiescent. miR-31 is present in quiescent satellite cells and inhibits *Myf5* translation [16]. In activated satellite cells, relative levels of miR-31 are reduced and *Myf5* protein accumulates, which initially requires translation. Furthermore, manipulation of miR-31 levels affects satellite cell activation and differentiation *ex vivo* and *in vivo* [16]. miR-489 is highly expressed in quiescent satellite cells and is rapidly downregulated during satellite cell activation. It induces satellite cell quiescence by suppressing the oncogenic protein *Dek*. *Dek* promotes the proliferative expansion of myogenic progenitors [17].

In human muscle, several miRNAs, including miR-106b, miR-25, miR-29c and miR-320c, are downregulated in quiescent satellite cells compared with proliferating satellite cells [18]. In the same study, miR-1, miR-133, miR-206 and miR-486 are identified to be involved in myotube formation. An elegant study by Marzi *et al.* [19] found that 21 miRNAs were induced during proliferation. These miRNAs, named proliferation-associated miRNA (PA-miRs), were repressed during differentiation. Another 35 miRNAs were induced during differentiation [named differentiation-associated miRNA (DA-miRs)]. Notably, some DA-miRs act as negative regulators of proliferation. Among the DA-miRs identified in muscle, seven miRNAs cause a reproducible reduction in proliferation (BrdU incorporation test): miR-1, miR-34, miR-22, miR-365, miR-29, miR-145 and Let-7.

MicroRNAs and signalling pathway

There are many signalling pathways through which miRNAs influence myogenesis and muscle metabolism. It is well known that insulin-like growth factor (IGF) signalling (Fig. 1) plays critical positive roles in skeletal muscle growth and myostatin activates pathways that suppress growth and/or induce muscle atrophy. Muscle-specific miR-1 modulates muscle cell growth and differentiation by targeting IGF-1. One study [20] demonstrated that miR-1 and IGF-1 protein levels are inversely correlated during C2C12 skeletal muscle differentiation because IGF-1 downregulates miR-1 expression by inactivating the FoxO3a transcription factor. In addition, transfection of miR-1 and miR-133a into neonatal rat cardiomyocytes abolished IGF-1-induced hypertrophy [21]. In another myomiR study [22], miR-206 directly regulates IGF-1 mRNA by targeting its 3'-UTR; reduction of miR-206 substantially increases IGF-1 mRNA level *in vivo*. Yet, another group of investigators found that the IGF-1 receptor (IGF-1R) is regulated by miR-133 [23]. They identified a conserved binding site for miR-133 in the 3'-UTR of IGF-1R. Increasing miR-133 in C2C12 cells significantly suppresses IGF-1R protein expression at the post-transcriptional level. Overexpression of miR-133 or knockdown of IGF-1R decreases the phosphorylation (i.e. activation) of Akt, the nexus of the IGF/PI3K/Akt signalling pathway. Another study [24] found that miR-125b negatively modulates myoblast differentiation and the levels of miR-125b decline during myogenesis. miR-125b targets IGF-II (IGF-2) in both myoblasts and regenerating muscles and is negatively controlled by the mammalian target of rapamycin (mTOR) [24].

A decrease in IGF-1 pathway activity accelerates muscle protein degradation by stimulating the ubiquitin proteasome system through activation of the FoxO transcription factors (FoxOs). Decreased phosphorylation of the FoxOs allows them to translocate to the nucleus where they direct the expression of a number of atrophy-inducing genes (i.e. atrogenes), including the muscle-specific E3 ligases, MAFbx/atrogenin-1 and Muscle RING-finger1 (MuRF1). miR-486 was reported to dampen FoxO1 and PTEN expression and their activities [25]. Our study using a miR-486 mimic showed that this miRNA blocked dexamethasone-stimulated protein degradation without influencing protein synthesis. miR-486 downregulated PTEN, thus increasing pAkt, resulting in decreased FoxO1 protein translation. Moreover, an increase in the level of miR-486 in muscles of mice with chronic kidney disease (CKD) resulted in suppression of MAFbx/atrogenin-1 and MuRF1 as well as an increase in muscle mass [26]. Dey *et al.* [27] also showed that miR-486 increased satellite cell differentiation by inhibiting Pax7. These observations demonstrate that miR-486 regulates several facets of the muscle atrophy programme and is therefore a potential therapeutic agent. miR-486 also decreased in the muscle of patients with Duchenne muscular dystrophy (DMD), and PTEN and FoxO1 are identified to be the target of miR486 [28]. However, in the same study, investigators also found that overexpressing miR-486 *in vivo* impaired muscle regeneration. This unexpective result could be due to the fact that miR-486 alters the cell cycle kinetics upon regeneration of muscle fibres [28]. Lastly, miRNA cluster miR-17–92 has been suggested to suppress PTEN expression. Overexpression of miR-17–92 in mice causes cardiac hypertrophy [29]; however, its function in skeletal muscle atrophy has not yet been studied.

Several studies demonstrate that miR-23a is an atrophy/hypertrophy-related miRNA, although the results are controversial. The MAFbx/atrogenin-1 and MuRF1 E3 ligases are prominently induced during muscle atrophy and mediate atrophy-associated changes in protein synthesis and degradation. Wada *et al.* [30] and others reported that miR-23a suppresses the translation of both MAFbx/atrogenin-1 and/or MuRF1 in a 3'-UTR-dependent manner [30,31]. Furthermore, they showed that ectopic expression of miR-23a is sufficient to protect muscles from atrophy *in vitro* and *in vivo*. Using miR-23a transgenic mice, they

found that miR-23a conveyed a resistance against glucocorticoid-induced skeletal muscle atrophy [30]. Our laboratory reported that miR-23a was one of nearly a dozen miRNAs that were decreased in the muscle of chronic renal failure mice [32]. This response is consistent with the well characterized increase in MAFbx/atrogen-1 and MuRF1 during atrophy [33]. Another study [31] in cardiac hypertrophy reports that miR-23a is a pro-hypertrophic miRNA. miR-23a expression was upregulated upon treatment with the hypertrophic stimuli including isoproterenol and aldosterone. The same group reported that nuclear factor of activated T cells (NFATc3) binds to the miR-23a promoter and increases its transcription. Recently, miR-23a was shown to inhibit myogenic differentiation. Luciferase reporter assays showed that miR-23a directly targets the 3'-UTRs of multiple adult fast myosin heavy chain (MHC) genes, including MHC-1, MHC-2 and MHC-4. Interestingly, the expression level of mature miR-23a is inversely correlated with myogenic progression in mouse skeletal muscle [34]. Moreover, an acute bout of endurance exercise reduced miR-23 expression by 84%. miR-23 is a negative regulator of peroxisome proliferator activated receptor-gamma coactivator-1 α (PGC-1 α). A decrease in miR-23 was associated with an increased expression of PGC-1 α mRNA and protein along with several downstream targets of PGC-1 α , including 5-aminolevulinate synthase (ALAS), citrate synthase (CS) and cytochrome c mRNA [35]. On the basis of computer-based searches, other muscle-related targets of miR-23 are myostatin and Ying Yang 1 (YY1), but they still need experimental validation [31].

Myostatin belongs to the transforming growth factor- β (TGF- β) superfamily and is well known to negatively regulate muscle growth. A recent study [36] found that miR-27a increases muscle cell proliferation by directly inhibiting myostatin. The myostatin 3'-UTR contains a putative recognition sequence for miR-27a and miR-27b that is conserved across a wide range of vertebrate species. In another study, miR-27b expression significantly attenuated the myostatin 3'-UTR-luciferase activity by approximately half. Mutation of the miR-27b recognition sequence significantly increased the activity of a myostatin 3'-UTR by approximately two-fold in C2C12 myotubes and myostatin mRNA degradation was decreased [37]. Inflammation cytokine TNF-related weak inducer of apoptosis (TWEAK) induced muscle atrophy by downregulation of miR-27a and miR-27b [38]. MyomiRs miRNA-1, miR-133 and miR-206 are significantly increased in myostatin knockout mice that have increased Myf5 and MEF2A expression [39].

Nonmuscle-specific microRNAs with diverse functions

miR-696 is considered to be a physical activity dependent miRNA. Microarray analysis for miRNAs in gastrocnemius muscle revealed that miR-696 was markedly upregulated by immobilization and downregulated by exercise [40]. miR-696 targets and inhibits PGC-1 α , and consistent with this, PGC-1 α protein is increased by exercise and decreased by immobilization (cast). This role of miR-696 was confirmed in cultured myocytes wherein elevation of intracellular miR-696 led to negative regulation of PGC-1 α protein along with the expression of mRNAs for downstream genes. In addition to its role in PGC-1 α regulation, miR-696-overexpression has been shown to decrease mitochondria biogenesis and fatty acid oxidation in transfected myocytes when compared with normal control myocytes [40].

miR-29 is a broadly expressed miRNA; however, emerging evidence suggests that miR-29 regulates myogenesis and muscle function. We and others have shown that miR-29 increases myoblasts differentiation by targeting YY1 [32], HDAC4 (histone deacetylase 4) [41] and Rybp (ring 1 and YY1-binding protein) [42], which are all negative regulators of myoblast differentiation. Working in nonmuscle systems, different groups have found that miR-29 negatively regulates IGF1, inhibits expression of the p85 subunit of PI3K, causes insulin

resistance and promotes apoptosis [43]. To date, these observations have not yet been reported in skeletal muscle.

In the past year, several miRNAs have been reported to play important roles in muscle development and regeneration. A new study [44] in pig muscle found that miR-378 promotes muscle development by directly regulating the 3'-UTR of bone morphogenetic protein 2 (BMP2) and mitogen-activated protein kinase 1 (MAPK1), both of which are important to myoblast proliferation and differentiation. Another study found that the level of miR-203b is negatively correlated with MyoD expression. This was further verified with a 3'-UTR luciferase reporter assay that proved a direct interaction between miR-203b and myoD [45]. In a murine muscle injury/regeneration model (cardiotoxin-induced muscle injury), investigators found that miR-351 promotes muscle progenitor cell proliferation and protects against apoptosis. Transcription factor E2f3, which plays a crucial role in the control of cell cycle and action of tumour suppressor proteins, has been identified to be the target of miR-351 [46]. In a drosophila muscle study, an miR-92b was shown to inhibit MEF2 (a key transcription factor for muscle development). The study also showed that miR-92b is activated by MEF2 and subsequently downregulates MEF2 through binding to its 3'-UTR. This relationship is important for maintaining the stable expression of both components during muscle development. The concept was proved by showing that deletion of miR-92b caused abnormally high MEF2 expression, leading to muscle defects and lethality. In addition, overexpression of miR-92b reduced MEF2 levels and caused muscle defects similar to those seen in MEF2 RNAi [47]. In another study, miR-26a was induced in an injury/regeneration animal model. Inhibiting miR-26a *in vivo* delayed muscle regeneration by upregulation of transcription factors Smad 1 and Smad 4 [48].

MicroRNA and muscle atrophy

Muscle atrophy can be divided into primary muscular disease, secondary muscular disorders and ageing sarcopenia. Primary muscle atrophy is caused by direct diseases of the muscle such as DMD. The secondary muscular disorders are disease related, which include CKD, sepsis, cancer, diabetes mellitus, heart failure and disuse related atrophies resulting from, that is, surgery, immobilization and space flight. In addition, exercise or other treatments (such as molecular silencing of myostatin) can prevent or reverse muscle atrophy [49]. Any miRNA influence on these diseases or procedures could impact muscle atrophy.

In primary muscular dystrophy, Eisenberg *et al.* [50] described 185 miRNAs that are upregulated or downregulated in 10 major muscular disorders in humans. There are five miRNAs (miR-146, miR-221, miR-155, miR-214, miR-222) that were found to be consistently altered in almost all samples analyzed. Greco *et al.* [51] showed that 11 miRNAs were altered in both DMD patients (DMD signature) and in MDX mice (DMD animal model). They found that regeneration miRNAs (miR-31, miR-34c, miR-206, miR-335, miR-449 and miR-494) were induced, degenerative miRNAs (miR-1, miR-29c and miR-135a) were downmodulated and inflammatory miRNAs (miR-222 and miR-223) were expressed in damaged muscle areas where their expression correlated with the presence of infiltrating inflammatory cells. Both of these studies indicate an important role of miRNAs in pathophysiological pathways that lead to primary muscle atrophy. Recently, a study found that miR-196a, delivered by an adeno-associated virus into a mouse model of spinal and bulbar muscle atrophy, ameliorated the associated atrophy. This muscle atrophy results from expansion of the poly Q tract of the androgen receptor. Delivery of exogenous miR-196a caused downregulation of the androgen receptor mRNA and reduced the disease-related muscle atrophy. This effect is accomplished through silencing of CUG binding protein (Elav-like family member 2) altering RNA splicing in these animals [52]. This study showed that disease-specific miRNA delivery may be a feasible therapeutic option.

Disease-related secondary muscle atrophy has been investigated by several laboratories. Inflammatory cytokines play an important role in the loss of skeletal muscle mass in various chronic diseases. TWEAK is a major muscle-wasting cytokine [8]. Results of a low-density miRNA array demonstrated that TWEAK inhibits the expression of several miRs, including miR-23b as well as muscle-specific miR-1-1, miR-1-2, miR-133a, miR-133b and miR-206 [38]. TWEAK induces skeletal muscle atrophy through inhibition of the PI3K/Akt signalling pathway and activation of the ubiquitin proteasome and nuclear factor kappa-light-chain-enhancer of activated B cells.

In patients with CKD, a decline in protein stores along with decreased muscle mass is associated with increased morbidity and mortality. Our laboratory examined a profile of miRNAs in muscles from mice with CKD and observed significant changes in 12 miRNAs [32], including decreased miR-23a, miR-29a and miR-29b. Chronic obstructive pulmonary disease (COPD) is another disease that is typified by muscle atrophy and carries with it a poor prognosis [53]. Assessment of lung and quadriceps function was performed in 31 patients with COPD and 14 healthy age-matched controls, along with measurement of daily activity. Analysis of a percutaneous quadriceps muscle biopsy showed a reduction in expression of miR-1 (2.5-fold) in patients compared with controls. Further correlations showed that miR-1 expression was associated with smoking history, lung function, fat-free mass index and percentage of type 1 fibres. miR-133 and miR-206 were negatively correlated with daily physical activity. IGF-1 mRNA was increased in the patients and miR-1 was negatively correlated with phosphorylation of Akt. Furthermore, the protein levels of HDAC4, another miR-1 target, were increased in the COPD patients [53].

Disused muscle atrophy has been studied in spaceflight animals. The investigators examined the gastrocnemius from mice flown on the 11-day, 19-h STS-108 shuttle flight and compared with the same muscle from normal gravity controls. The microarray data revealed that 272 mRNAs were significantly altered by spaceflight. In addition, the muscle fibre type shifts from slow twitch to the faster muscle fibre types. The majority of results observed in the space flight muscles were confirmed using the hind-limb suspension disuse model and these responses were reversed upon reloading (return to use) of the muscle. Among the data from the space flight study were some potentially contradictory observations. For example, several mRNAs altered by spaceflight were associated with muscle growth, including PI3K/p85 α , insulin response substrate-1 (IRS-1), FoxO1 and MAFbx/atrogen1. In addition, myostatin mRNA (pro-atrophy factor) expression tended to increase and mRNA levels of the myostatin inhibitor Follistatin-related protein 3 (FSTL3) tended to decrease [54], resulting in loss of muscle mass. Disused muscle atrophy was also tested in 12 young, healthy male animals that completed 7 days of bed rest with vastus lateralis muscle biopsies. Bed rest reduced skeletal muscle miR-1 and miR-133a content approximately 10% [55]. Denervation belongs to the disused muscle family of atrophies. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by loss of motor neurons, denervation of target muscles, muscle atrophy and paralysis. miR-206 is dramatically increased in a mouse model of ALS, possibly in an attempt to correct the denervation of the muscles. It has been suggested that elevation of this miRNA may serve as a corrective therapy for the denervation. Consistent with this, exogenous addition of miR-206 has been shown to slow ALS progression by promoting the compensatory regeneration of neuromuscular synapses. miR-206 mediates these effects at least in part through HDAC4 and fibroblast growth factor signalling pathways [56].

Ageing sarcopenia refers to the gradual decline in muscle mass and muscle quality that occurs with ageing. The impact of miRNA has been studied in muscle biopsy samples from 17 old and 19 young men [57]. Eighteen miRNAs were differentially expressed in older humans. Let-7 family members, Let-7b and Let-7e, were significantly elevated. Functional

and network analysis determined that gene targets of the Let-7s were associated with cell cycle control such as cellular proliferation and differentiation signal pathways. In addition, Pax-7 mRNA expression was lower in older individuals. These data suggest that ageing is characterized by a higher expression of Let-7 family members that may downregulate genes related to cellular proliferation [57].

Insulin and diabetes

Insulin is the major hormone controlling whole-body energy homeostasis and is also involved in the regulation of miRNA expressions in human skeletal muscle. One group carried out comparative miRNA expression profiles in human skeletal muscle biopsies before and after a 3-h euglycemic-hyper-insulinemic clamp using TaqMan low-density arrays. miR-1 and miR-133 are upregulated after the clamp. Insulin downregulates the expressions of 39 distinct miRNAs in human skeletal muscle. In-silico analysis indicates that these miRNAs are correlated with potential target mRNAs for proteins that were mainly involved in insulin signalling and ubiquitination-mediated proteolysis [8]. Insulin resistance also impacts muscle atrophy. One study showed that miR-24 is downregulated in skeletal muscle from Goto-Kakizaki type II diabetic rats and miR-24 directly targets p38 MAPK [58]. As p38 MAPK is related with insulin resistance and muscle wasting, their finding that 'a decreased miR-24 results in rising p38 MAPK' suggests that decreasing miR-24 may cause insulin resistance. miR-144 has also been demonstrated to cause insulin resistance by inhibition of IRS-1 at both mRNA and protein levels, as well as through a direct interaction with 3'-UTR of IRS-1 [59]. The role of miRNAs and diabetes is the subject of a recent comprehensive review [60].

Essential amino acids (EAAs) stimulate muscle protein synthesis in humans and are also involved with miRNA regulation. Muscle biopsies were obtained from the vastus lateralis of seven young adult participants before and 3 h after ingesting 10 g of EAA. Following EAA ingestion, miR-499, miR-208b, miR-23a and miR-1 increased. The muscle growth genes *MyoD1* and *FSTL1* mRNA expression increased, and myostatin and MEF2C mRNA were significantly downregulated. Although these data point to changes in expression levels of these proteins, proof that miRNA directly regulates the protein expressions has not yet been experimentally validated [8].

CONCLUSION

Current research in miRNAs is built upon earlier studies. Although initial studies identified new miRNAs, current work emphasizes the role that these identified miRNAs play in regulation of muscle function or fidelity. Although new miRNAs are still being identified that are important to muscle health, much of the research published in the past couple years has investigated the changes in miRNAs and related proteins in diverse muscle disorders and diseases. Although many miRNA targets have been validated, our current understanding in this area is still limited. Studies have emphasized identifying miRNAs and correlating their abundance with potential targets. Studies that have focused on miRNA function are much fewer and there is a great need to decipher the precise miRNA signatures for muscle atrophy. In the immediate future of miRNA study, some questions should be considered: Does the miRNA change in a given disease model? What is the function of this miRNA? Which proteins are directly targeted by this miRNA? How is this miRNA delimited: by transcriptional regulation (does a transcription factor promote/inhibit production?) or by increasing/decreasing degradation? Can you manipulate this miRNA to prevent a disorder or disease for translation study? Clearly, miRNAs are intimately involved in all aspects of muscle physiology and a better understanding of the miRNA regulation pathways in muscle

atrophy will enhance our ability to develop miRNA-based biomarkers for diagnosis and therapy.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 353–354).

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KEY POINTS

- Seven differentiation-associated microRNAs act as negative regulators of muscle cell proliferation.
- Insulin-like growth factor (IGF) signalling is negatively regulated by miR-1, miR-133, miR-206, miR-125b, miR-486 and miR-17-92 downregulate Phosphatase and tensin homolog (PTEN), thus increasing pAkt. miR-23a suppresses the translation of both MAFbx/atrogen-1 and MuRF1, thus directly inhibiting muscle atrophy.
- miR-696 is considered to be a physical activity dependent miRNA and translationally regulates PGC-1 α (peroxisome proliferator activated receptor-gamma coactivator-1 α) by altering mitochondrial biogenesis.
- A decrease in miR-23 (an atrophy/hypertrophy-related miRNA) is associated with an increase in PGC-1 α (a regulator of mitochondrial biogenesis and function) mRNA and protein in muscle.
- miR-29 regulates myogenesis by increasing muscle cell differentiation, and possibly by decreasing muscle cell proliferation.

FIGURE 1.

MicroRNA regulation of IGF/PI3K/Akt signalling: Muscle-specific miR-1 regulates IGF/PI3K/Akt signalling by directly targeting IGF-1. The activated state of the IGF-1 reciprocally regulates miR-1 expression through the FoxO transcription factor. FoxO binds on miR-1 promoter region and increases transcription of miR-1. miR-206 and miR-133 also target IGF-1. miR-133 has a conserved and functional binding site in the 3'-UTR of IGF-1R, which results in decreased IGF-1R abundance. miR-125b targets IGF-2. miR-486 and miR-17-92 downregulate PTEN, thus increasing pAkt which, in turn, phosphorylates FoxO resulting in its inactivation. Inactivation of FoxO limits muscle wasting. miR-23a suppresses the translation of both atrogin-1 and MuRF1 in a 3'-UTR-dependent manner, thus directly inhibiting muscle atrophy.