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Potential Role of MicroRNA in the Anabolic Capacity of Skeletal Muscle With Aging

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MARGOLIS, L.M. and D.A. RIVAS. Potential role of microRNA in the anabolic capacity of skeletal muscle with aging. *Exerc. Sport Sci. Rev.*, Vol. 46, No. 2, pp. 86–91, 2018. *Age-induced loss of skeletal muscle mass and function, termed sarcopenia, may be the result of diminished response to anabolic stimulation. This review will explore the hypothesis that alterations in the expression of microRNA with aging contributes to reduced muscle plasticity resulting in impaired skeletal muscle adaptations to exercise-induced anabolic stimulation.*

Key Words: myomiR, sarcopenia, exercise, anabolic resistance, miR

KEY POINTS

- microRNA are small noncoding RNA that regulate skeletal muscle mass by targeting.
- In young, healthy individuals, skeletal muscle microRNA are inversely associated with rates of skeletal muscle protein synthesis after exercise.
- Aging blunts response of microRNA expression to resistance exercise, resulting in impaired skeletal muscle adaptations to exercise-induced anabolic stimulation.
- Circulating microRNA profiles reflect *anabolic resistance* in skeletal muscle with aging.

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INTRODUCTION

It is well established that mechanical load placed on skeletal muscle during resistance exercise activates anabolic intracellular signaling processes, increases the rate of muscle protein synthesis, and, with repeated exposure, (*i.e.*, training) leads to gains in muscle mass (1). With aging, there is insufficient skeletal muscle plasticity, resulting in a blunted rate of skeletal muscle protein synthesis (*i.e.*, anabolism) after acute exposure to potent anabolic stimuli, such as resistance exercise (2–4). Diminished anabolism with aging has been termed *anabolic resistance* (5). Failure to preserve normal anabolic processes while proteolytic (*i.e.*, breakdown) mechanisms are maintained or upregulated with aging results in development of sarcopenia — age-associated decline in skeletal muscle mass and function (5,6). Over time, the progressive loss in muscle mass can compromise an individual's quality of life, leading to loss of independence and diminished healthspan.

To minimize declines in skeletal muscle mass and mobility with aging, an understanding of the underlying molecular processes regulating anabolism is crucial to determine potential therapeutic targets. Recently, our group (7) has identified dysregulation of microRNA (miRNA; small noncoding RNA, approximately 18–25 nucleotides in length) with aging as a potential mechanism governing the adaption within skeletal muscle in response to anabolic stimulation. Specifically, divergent responses in miRNA expression between younger and older individuals after a bout of resistance exercise showed impairment of anabolic signaling with aging (7). In addition, we have reported that this blunted anabolic response to resistance exercise in skeletal muscle with aging also can be observed in discordant expressions of circulating (serum) miRNA (c-miRNA) profiles (8).

This review will provide a contemporary overview of the biogenesis and function of miRNA with specific focus on the role of miRNA in governing biological processes involved in skeletal muscle anabolism and catabolism. Based on work from our laboratory, the purpose of this review is to examine the hypothesis that alterations in the expression of miRNA with aging contributes to reduced muscle plasticity resulting in impaired adaptations to exercise-induced anabolic stimulation. In addition, we will explore the hypothesis that circulating miRNA can be used as a noninvasive marker reflective of age-related anabolic resistance after resistance exercise.

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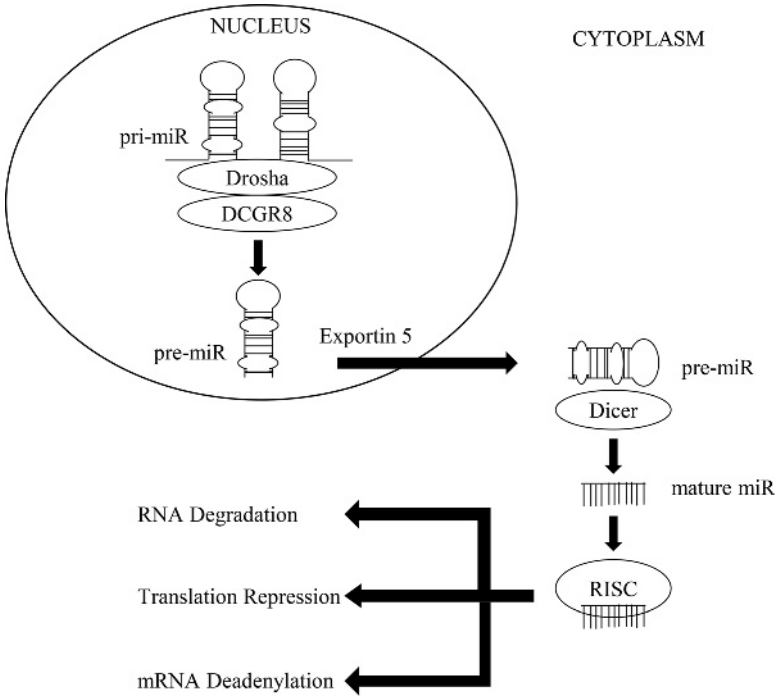

Figure 1. microRNA Biogenesis; Biogenesis of miRNA begins in the nucleus of the cell, where RNA polymerase II transcribes primary-miRNA (pri-miRNA), consisting of thousands of nucleotides with stem-loop structures. The enzyme Drosha, a member of the ribonuclease (RNase) III superfamily of double-stranded RNA-specific endoribonuclease, together with DiGeorge syndrome critical region gene (DGCR8), cleaves the stem-loop structure of the pri-miRNA to form precursor miRNA (pre-miRNA). Conversion of pri-miRNA to pre-miRNA is a critical step because it is site specific, dictating the sequence of the mature miRNA. The pre-miRNA translocates out of the nucleus into the cytoplasm by small RNA transporter Exportin 5, which is a GTP-dependent process. Once in the cytoplasm, pre-miRNA is processed by Dicer, another enzyme in the RNase III family, to form mature miRNA. One strand of the mature miRNA is bound by Argonaute, a protein that directly binds to miRNA, forming a protein complex called RISC, allowing miRNA to bind to target mRNA, resulting in posttranscriptional modifications that repress the translation of protein.

MICRORNA BIOGENESIS AND FUNCTION

Biogenesis of miRNA begins in the nucleus of the cell (Fig. 1), where it is processed and translocated into the cytoplasm to form a mature miRNA (9). The mature miRNA is bound by Argonaute, forming a protein complex called RNA-induced silencing complex (RISC) (10). This RISC complex allows miRNA to bind to target mRNA, resulting in posttranscriptional modifications that repress the translation of protein (11). Through this mechanism of negative inhibition, miRNA regulate gene expression. miRNA-dependent gene regulation is a complex process because one miRNA can regulate hundreds to thousands of genes (12). The ability for one miRNA to inhibit the expression of a large number of genes allows a single miRNA to repress several mRNA in a common biological pathway, resulting in robust regulation of an entire molecular process (13). In addition, one gene can be targeted by multiple miRNA, resulting in cooperative/redundant regulation of a signal molecular process (12). Through these mechanisms of regulation, miRNA have a critical role in the development and maintenance of physiological processes that determine muscle fiber number, type/phenotype, and mass/size (14).

MICRORNA REGULATION OF SKELETAL MUSCLE ANABOLISM

Skeletal muscle is highly enriched with specific microRNA (miR-1, miR-133a, miR-133b, miR-206, miR-208, miR-221, miR-222, miR-486, miR-499) that have together been termed myomiRs (14,15). Although the exact mechanisms remain unclear, in young, healthy individuals, miRNA expression is acutely altered by anabolic stimulation (7,16–18). In general, after resistance exercise, there is a downregulation in miR-1, miR-133a, miR133b, and miR-206 expression (16,19), whereas more metabolically demanding endurance exercise results in an upregulation or no change in expression of these miRNA (19–21). Divergent response in miRNA expression to various exercise modes seems to be due, at least in part, to miRNA being sensitive to alteration in the rate of muscle protein synthesis, with miR-206 and miR-499 expression reported to be inversely associated with muscle protein synthesis during exercise (19). Given that miRNA function through negative inhibition, concurrent reductions in miRNA expression with increased muscle protein synthesis rates suggest a potential feed-forward mechanism, where acute anabolic stimulation downregulates the inhibition of miRNA to initiate training

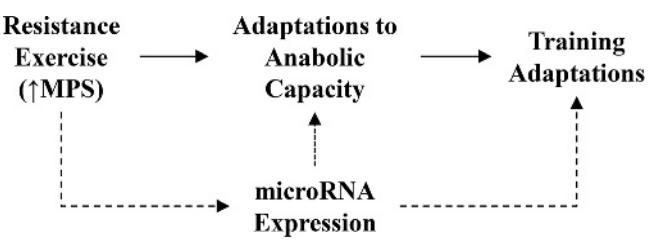

Figure 2. Schematic of hypothesized feed-forward mechanism of elevated muscle protein synthesis (MPS) rates modulation microRNA expression to aid in regulation of training adaptations.

adaptations through enhanced translation of proteins regulating skeletal muscle anabolism (Fig. 2).

There is a growing body of evidence that miRNA have a significant impact on skeletal muscle growth, with several miRNA participating in the regulation of signaling proteins involved in muscle protein synthesis (mechanistic target of rapamycin; mTORC1) and breakdown (factor forkhead box O 1; FOXO1) signaling cascades (Fig. 3). In muscle, miR-1, miR-133a-3p, and miR-199a-3p target insulin growth factor (IGF)-1 and IGF-1R, blunting rates of protein synthesis (22,23). During periods of muscle growth, induced by mechanical load, miR-1 and miR-133a expression is downregulated to allow for activation of mTORC1 signaling through IGF-1, resulting in increased rates of protein synthesis (24). In muscle atrophy, miR-199a-3p expression is increased (25), with this overexpression resulting in impairment of muscle hypertrophy, diminishing phosphorylation of Akt and mTORC1 (26). In addition, miR-99a, miR-99b, and miR-100-5p influence cellular growth by both directly and indirectly mediating translation of mTORC1. Specifically, increased expression of miR-99a and miR-99b inhibit transcription of mTOR, whereas miR-100-5p targets both Akt and mTOR, resulting in diminished total protein content and hypertrophy (27,28). Furthermore, it is possible that miRNA play an important role in the shifting of intracellular signaling from catabolism to anabolism. Expression of the upstream inhibitor of Akt, phosphatase and tensin homolog (PTEN), is diminished by miR-221, miR-222, and miR-486 to promote cellular growth (29,30). The miR-17 ~ 92 cluster, which contains seven miRNA, also may participate in alterations in Akt-mTOR signaling. Similar to miR-221 and miR-222, miRNA in the miR-17 ~ 92 cluster (miR-17-5p, miR-19a-3p, and miR-19b-3p) inhibit PTEN, promoting Akt-mTOR signaling (31). Increased Akt activity due to alterations in miRNA expression may not only promote synthesis, but diminish protein breakdown as well, resulting in a positive protein balance. Upregulation of miR-486 activates Akt and diminishes FOXO1 protein expression. Diminished FOXO1 protein content by miR-486 results in reductions in transcription of atrophy proteins MAFbx and MuRF1, potentially minimizing muscle protein breakdown (29). In addition, miRNA, particularly miR-1, miR-133a, miR-133b, and miR-206, can further regulate skeletal muscle mass through control of transcription factors governing myogenesis/regeneration (15). Given that the majority of work in altered miRNA expression with aging has focus on anabolism, regulation of miRNA on myogenesis is outside the scope of this review. For a review of miRNA regulation of myogenesis see Kirby *et al.* (32)

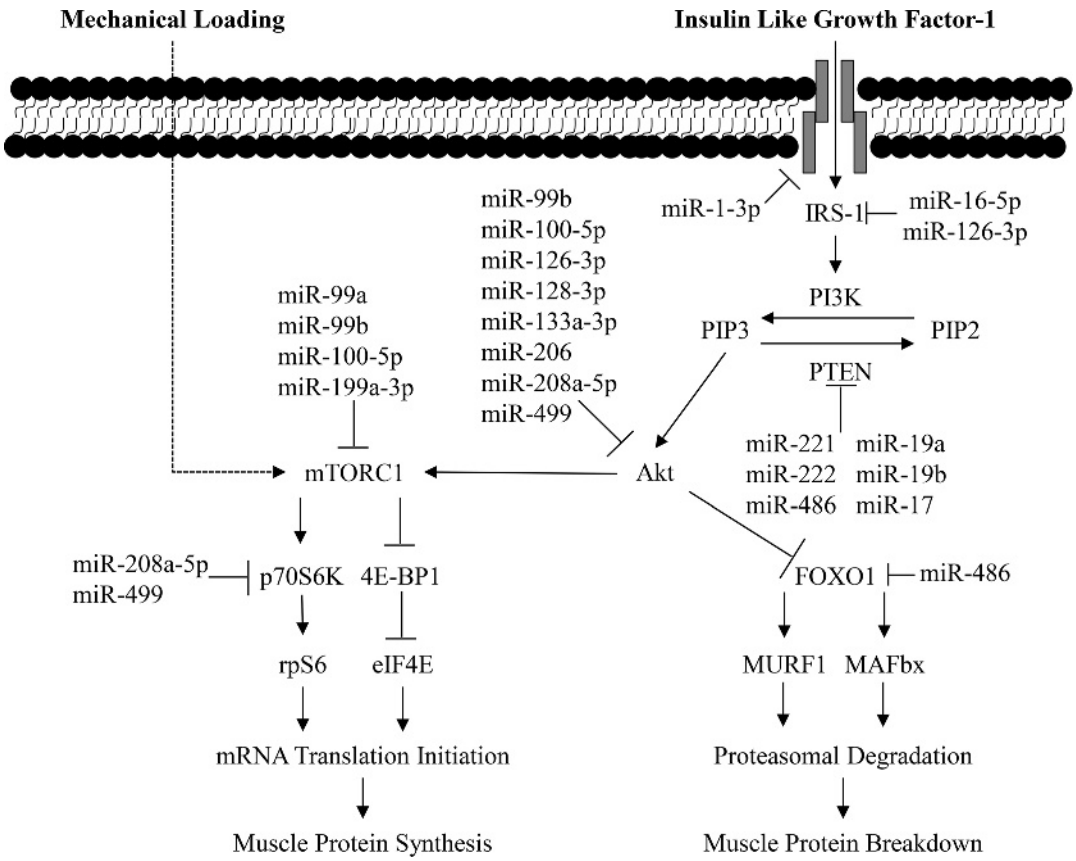

Figure 3. Interaction between microRNA and intracellular signaling pathways regulating skeletal muscle protein synthesis and breakdown. Activation of mechanistic target of rapamycin (mTORC1) triggers downstream signaling through p70 ribosomal S6 kinase (p70 S6K), ribosomal protein S6 (rpS6), eukaryotic elongation factor 2 kinase (eEF2), and eukaryotic initiation factor 4E-binding protein (4E-BP1), increasing mRNA translational efficiency and muscle protein synthesis. Muscle protein breakdown from ubiquitination results through the muscle-specific E3 class of ubiquitin ligases, atrogin-1/muscle atrophy F-box (MAFbx), and muscle RING finger-1 (MuRF1). Activity of atrogin-1/MAFbx and MuRF1 are regulated by the FOXO family of transcription factors, which when dephosphorylated, translocate to the nucleus to mediate increased expression of these ubiquitin ligases. The ubiquinated proteins are transferred to the 26S proteasome for subsequent degradation.

AGING AND SKELETAL MUSCLE MICRORNA EXPRESSION

There have been a limited number of investigations examining the potential role of modulation in miRNA expression on age-associated declines in skeletal muscle anabolism (7,16,33). After performance of knee extension exercise fixed at 70% of participant's one-repetition maximum and ingestion of 20 g essential amino acid (EAA), expression of miR-1 was reduced in young participants, with no change observed in older individuals (16). A divergent response in miR-1 expression with aging likely indicates a lack of an anabolic response to the bout of resistance exercise. Reductions in miR-1 expression during periods of hypertrophy suggest potential activation of the mTORC1 pathway through IGF-1 signaling because miR-1 inhibits IGF-1 in skeletal muscle (22). In agreement with these findings, our laboratory (7) recently observed that after a single bout of resistance exercise, expression of 60 miRNA assessed in skeletal muscle was not altered in older participants, whereas younger participants experienced a significant reduction in the expression of 16 of these 60 miRNA. The absence of exercise-induced miRNA regulation with aging was accompanied by a blunted gene transcription response and diminished activation of the mTORC1 signaling cascade compared with younger participants (3). Impairment of resistance exercise–induced alterations in skeletal muscle miRNA and mRNA expression, as well as diminished phosphorylation of mTORC1 signaling with aging, suggests a potential link governing anabolic resistance.

To further examine whether altered miRNA expression was a potential mechanistic target for diminished muscle mass with aging, principal component analysis (PCA) was conducted on miRNA with differing expressions between younger and older subjects to determine which miRNA distinguished aging (7). This analysis identified miR-126-3p as a potential target influencing the divergent anabolic response to resistance exercise with aging. To test the role of miR-126-3p on regulation of molecular pathways controlling skeletal muscle anabolism, *in vitro* analysis was performed, manipulating the expression of miR-126-3p through transfection of miR-126-3p inhibitor or mimetic for 24 h in myocytes and myotubes. Inhibition of miR-126-3p protein content of insulin receptor substrate 1 increased 50%, whereas FOXO1 decreased 25% compared with control myocytes. In addition, when miR-126-3p was overexpressed, myogenic regulators MyoD and Myf5 were 60% and 50%, respectively, lower compared with controls. After 30 min exposure to IGF-1 in myotubes, upregulation of p-Akt^{Ser473} was greater in miR-126-3p inhibited myotubes compared with control. Similarly, a downstream target of mTORC1, p-rpS6$^{Ser240/244}$ was activated to a greater extent in miR-126-3p-inhibited myotubes compared with controls. Together, findings from *in vivo* and *in vitro* analysis identify miR-126-3p dysregulation with aging as a novel regulator suppressing skeletal muscle regeneration and growth after exercise-induced adaptations within skeletal muscle.

In agreement with findings from our laboratory, high-throughput analysis of 754 miRNA identified 26 miRNA that were differentially expressed with aging in response to resistance exercise or a combination of the two (17). Top cellular functions of these miRNA were determined using ingenuity pathway analysis. This bioinformatics analysis revealed that six (miR-99a-5p, miR-99b-5p, miR-100-5p, miR-149-3p, miR196b-5p, and miR-199a) of these 26 miRNA were validated to target proteins within the Akt-mTORC1 signaling cascade. As described previously, members of the miR-99/100 family are of particular interest because these miRNA directly target mTOR to suppress protein synthesis and anabolism (27,28). Specifically, after acute resistance exercise, miR-99b-5p and miR-100-5p expression were diminished in young but not old participants. Again, the lack of response in expression of miRNA associated with regulation of anabolic signaling proteins with aging in skeletal muscle indicate dysregulation in miRNA expression after acute anabolic stimulus may contribute to age-associated declines in skeletal muscle mass.

CIRCULATING MICRORNA

Although miRNA have been shown to function in the cell where they are transcribed, recently, it has been reported that an alternative fate exists, where rather than remaining in the cytoplasm of the cell, miRNA can be packaged and exported into the circulation (c-miRNA) (34). Presence of miRNA in the circulation can be the result of multiple mechanisms and transporters. Within the cytoplasm, membrane-derived vesicles (exosomes and microvesicles) can take up premature and mature miRNA, where they can then be released into the circulation to be transferred to recipient cells (34). In addition to exosomal and microvesicle transportation, c-miRNA are actively transported in RNA-binding protein (Argonaute2), as well as high-density lipoproteins (high-density lipoprotein and low-density lipoprotein) (35,36). Furthermore, miRNA can be present in circulation passively in apoptotic bodies that have been shed by tissues (37). Once released into circulation, c-miRNA can be taken up by recipient cells to inhibit transcription of target genes (35). The mechanism involved in the uptake of exosome-bound c-miRNA by recipient cells remains elusive. However, it has been suggested that miRNA may be removed from circulation by endocytosis, fusion to the plasma membrane, scavenger receptor uptake, or interaction at the cellular surface to alter intracellular signaling (38).

Although the field of c-miRNA research is relatively new, since 2011, more than 30 manuscripts have been published reporting the influence of acute and chronic exercise on alterations in the expression of c-miRNA profiles, which have recently been described in a review by Sapp *et al.* (39). Together, these manuscripts clearly show that c-miRNA profiles can be altered by a bout of exercise or training (39). Furthermore, although only a small number of studies have been conducted, modulation in c-miRNA expressions seems to be sensitive to exercise modality (40,41) and fitness level/training mode (42,43). Exercise has been well-established to alter physiological processes within multiple tissues, thus adaptations in c-miRNA expression in response to acute exercise stimulation and training status indicates that c-miRNA profiles may be reflective of the underlying physiological status at the cellular level. In addition, because c-miRNA can participate in cell-to-cell communication (35), modulation of expression profiles may indicate a functional role in governing training adaptations. The application of c-miRNA as a noninvasive marker of skeletal muscle adaptations to exercise may be of particular interest in aging research because attainment of muscle samples, especially in older frail individuals, can be difficult because of lower amounts of the tissue and high infiltration of intermuscular fat.

INFLUENCE OF AGING ON CIRCULATING MICRORNA EXPRESSION AFTER RESISTANCE EXERCISE

Recent findings from our laboratory (8) reported that aging results in a divergent response in c-miRNA expression after a bout of resistance exercise. Of 90 c-miRNA assessed, 25 c-miRNA were altered by aging or resistance exercise. Using principle component analysis to group c-miRNA, 10 c-miRNA (miR-19b-3p, miR-193-5p, miR-19a-3p, miR-106-5p, miR-20a-5p, miR-17-5p, miR143-3p, miR-26b-5p, miR-18a-5p, and miR-93-5p) were identified to explain the majority of the variance within the dataset. After resistance exercise, expression of all 10 c-miRNA were increased in younger participants and decreased in older participants. Functional analysis was then performed assessing interactions between c-miRNA-to-mRNA expressions in skeletal muscle (7) using IPA. Outcomes of IPA revealed an absence of an anabolic response to resistance exercise with aging because markers of anabolic signaling, IGF-1 and mTOR, were identified as top canonical pathways in younger, but not older, participants. Further strengthening the bioinformatics data from this investigation, positive associations were observed between the expressions of miR-19a-3p, miR-19b-3p, miR-20a-5p, miR-26b-5p, miR-143-3p, and miR-195-5p to the phosphorylation status (*e.g.*, activity) of p-Akt^{Ser473} and p-p70S6K^{Thr389}. This finding suggests that increased expressions of these c-miRNA may be indicative of an anabolic response within skeletal muscle. Coupling state-of-the-art integrative analytics with findings from traditional bench top techniques strengthen that c-miRNA can be used as noninvasive markers to predict adaptations reflective of molecular processes in skeletal muscle to acute resistance exercise with aging.

Interestingly, 7 of the 10 c-miRNA (miR-17-5p, miR-18a, miR-19a, miR-19b, miR-20a, miR-93, and miR-106b) identified by our PCA results are members of the miR-17 ~ 92 cluster or extended miRNA families (31,44). Clustering of miRNA indicate that they are generated from a primary transcript and have a large overlap in their sequences and, thus, function (44). As described in the "microRNA Regulation of Skeletal Muscle Mass" section, members of the miR-17 ~ 92 cluster have shown convergence of these miRNA on Akt-mTORC1 signaling within tissue (45). A main target of these miRNA is PTEN, an inhibitor of the PI3K-Akt pathway. Inhibition of PTEN can promote cellular survival and proliferation through increased activation of Akt-mTORC1 signaling (31). Identification of divergent c-miRNA profiles that are members of the same family of miRNA–and share similar target genes–after resistance exercise enhances the potential use of c-miRNA as potential predictive markers of resistance exercise-induced adaptations because a single miRNA can target hundreds of different genes.

FUTURE DIRECTION OF RESEARCH: EXOSOMAL-MIRNA AS SIGNAL TRANSDUCERS IN INTERCELLULAR COMMUNICATION

Exosomes, small extracellular membrane vesicles with the size range of 40–100 nm that are formed by exocytosis of multivesicular endosomes, contribute to multiple aspects of physiology, metabolism, and disease, including communication between cells (46). Exosome can be released from multiple cell types, including skeletal muscle, and contain proteins, lipids, DNA, RNA, and importantly, miRNAs (47). Given that release of exosomes from cells would indicate an active process by which c-miRNA may participate in cell-to-cell communication, determining how select miRNAs are transported in exosomes to target organs could illuminate the function of altered c-miRNA profiles and improve our understanding and application of therapeutic approaches aimed at maintaining or improving muscle health.

Although presently, not much is known regarding alterations in exosome-derived c-miRNA in humans, cell culture and rodent models suggest that exosomes carrying specific miRNAs, such as miR-1, miR-21, miR-133, miR-182, and miR-206, are targeted to myocytes and modulate the physiology and pathology status of myocytes by altering gene expression (48,49). We hypothesize that miRNA carried in exosomes have essential roles as signal transducers that trigger the adaptations of muscle and other organs such as the adipose tissue and liver to maintain homeostasis. Indeed, miRNAs shuttled between cells are shown to be preserved and mediated by microvesicles including exosomes, which are emerging as potent promoters of genetic transfer (50). Recent work had highlighted that extracellular vesicles are able to efficiently deliver their parental cell-derived molecular cargo to target cells, resulting in structural changes at the RNA, protein, or even the phenotypic level (34). These data provide evidence for the importance of understanding the role of exosomes and their cargo in adaptation of skeletal muscle with age, exercise, and chronic disease. For these reasons, exosomes have recently gained major scientific interest as a therapeutic application for a drug delivery system. Conceivably, determining the essential miRNA cargo packaged in exosomes may reveal crucial miRNAs for targeting skeletal muscle in an attempt to mitigate sarcopenia.

CONCLUSIONS

In conclusion, findings from our laboratory (7) and others (16,17) show that in response to resistance exercise, modulation in skeletal muscle miRNA expression is blunted with aging. In addition, we have reported that discrepancies in miRNA expression with aging also are present in circulation, with differences in c-miRNA profiles reflective of anabolic resistance (8). Alterations in miRNA expression may be part of a feed-forward mechanism stimulating skeletal muscle growth. Elevations in the rate of skeletal muscle protein synthesis after resistance exercise stimulate downregulations in specific microRNA to diminish translation inhibition of proteins within the mTORC1 signaling cascade (19). As such, discordant responses in skeletal muscle and circulating miRNA expression to resistance exercise may be a potential mechanism blunting skeletal muscle plasticity and ultimately resulting in age-associated declines in skeletal muscle mass and function (*i.e.*, sarcopenia).

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