Regulation of muscle atrophy by microRNAs

REVIEW

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Regulation of muscle atrophy by microRNAs: 'AtromiRs' as potential target in cachexia

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Purpose of review
To provide an overview and describe the mode of action of miRNAs recently implicated in muscle atrophy, and discuss the challenges to explore their potential as putative therapeutic targets in cachexia.

Recent findings
Recent work showed differentially expressed miRNAs in skeletal muscle of patients with cachexia-associated diseases. Studies using experimental models revealed miRNA regulation of the anabolic IGF-1 and catabolic TGF-β/myostatin pathways, and downstream protein synthesis and proteolysis signaling in control of muscle mass.

Summary
Cachexia is a complex metabolic condition associated with progressive body weight loss, wasting of skeletal muscle mass and decrease in muscle strength. MiRNAs play a central role in post-transcriptional gene regulation by targeting mRNAs, thereby coordinating and fine-tuning many cellular processes. MiRNA expression profiling studies of muscle biopsies have revealed differentially expressed miRNAs in patients with low muscle mass or cachexia. Evaluation in experimental models has revealed muscle atrophy, inhibition of protein synthesis and activation of proteolysis in response to modulation of specific miRNAs, termed 'atromiRs' in this review. These exciting findings call for further studies aimed at exploring the conservation of differentially expressed miRNAs across diseases accompanied by cachexia, identification of miRNA clusters and targets involved in muscle atrophy, and probing whether these miRNAs might be potential therapeutic targets for cachexia.

Keywords
atromiRs, cachexia, microRNAs, myomiRs, skeletal muscle atrophy

INTRODUCTION

Cachexia is a complex metabolic condition associated with progressive body weight loss, wasting of skeletal muscle mass and decrease in muscle strength. A plethora of factors and biological mechanisms are involved in the pathophysiology of cachexia. Here, we discuss recent work on the involvement of microRNAs (miRNAs) in the regulation of skeletal muscle mass and speculate on their potential as therapeutic targets to modulate cachexia.

CACHEXIA

Cachexia is highly prevalent in patients with certain types of cancer and advanced stages of diseases such as chronic obstructive pulmonary disease (COPD), chronic kidney disease (CKD) and heart failure. The most distinct characteristics of cachexia are progressive body weight loss and wasting of skeletal muscle. Muscle wasting is an important determinant of physical disability, diminished quality of life, and is associated with increased mortality. In addition, cachexia limits therapeutic options and efficacy as cachectic patients display reduced tolerance and responsiveness to interventions, as exemplified by dose-limiting toxicity of radiation therapy and chemotherapy in cachectic cancer patients [1].

At the tissue level, loss of myofibrillar protein in muscle fibers, that is, decrease in size and not

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KEY POINTS

- MiRNA profiling studies have identified differentially expressed miRNAs in muscle biopsies of cachectic patients.
- Using experimental models, miRNAs were shown to play a critical role in the regulation of skeletal muscle atrophy.
- Further identification of putative 'atromiRs', and elucidation of their regulation and mode of action in muscle atrophy will contribute to our understanding of cachexia, and allow probing their potential as therapeutic targets in cachexia.

reduction in the number of myofibers, is one of the hallmarks of cachexia. The reduction in protein content is the consequence of both decreased protein synthesis and increased degradation (proteolysis). The proximal pathways that control protein synthesis and proteolysis are well described during acute changes in muscle mass. In addition, accretion of muscle progenitor (satellite) cell-derived nuclei into adult myofibers has been implicated in muscle mass homeostasis [2]. However, the exact cellular mechanisms underlying altered regulation of protein and myonuclear turnover and the triggers initiating atrophy signaling in skeletal muscle in cachexia remain to be identified.

MICRORNAS, BIOGENESIS, AND MODE OF ACTION

MiRNAs are small, ±22 nucleotides (nt) long single-stranded RNAs that play a central role in post-transcriptional gene regulation by targeting mRNAs, thereby coordinating and fine-tuning many cellular processes. The human genome encodes more than 1000 miRNAs, regulating the expression of at least 50% of all human genes. These pivotal post-transcriptional regulators of gene expression themselves are regulated in a transient and tissue-specific manner during development, homeostasis, and disease [3].

MiRNAs are derived from protein-coding intragenic or non-coding intergenic regions of the DNA and are often found in clusters. A cluster of miRNAs is defined as several miRNA genes with high-sequence homology located on the same chromosomal locus, resulting in their coordinated regulation. The observed high-sequence homology between the miRNAs in a cluster confers activity towards mRNA targets of genes that operate in the same cellular pathway, allowing a miRNA cluster to regulate various components of the same biological process [4]. MiRNA genes are first transcribed as long primary transcripts referred to as primary miRNA (pri-miRNA). Two serial processing reactions take place to obtain mature miRNA. The first reaction occurs in the nucleus and is executed by the microprocessor complex. The stem-loop structure is cleaved by the nuclear RNase III Drosha to produce an approximately 70nt hairpin structure, termed precursor miRNA (pre-miRNA). The pre-miRNA is subsequently transported to the cytoplasm, where it is cleaved by a second RNase III enzyme (Dicer) to yield an approximately 22nt miRNA duplex, called mature miRNA. To exert their regulatory function, one strand of this duplex, the so-called miRNA guide strand (5p), associates with Argonaute 2 proteins to form the miRISC complex (miRNA-induced silencing complex). This miRISC complex is directly responsible for gene silencing either via mRNA degradation or translational repression. The other strand, indicated as passenger strand (3p), is thought to be degraded (3).

Individual miRNAs may engage with multiple mRNA targets, often encoding various components within the same intracellular network. Typically, the miRNA recognizes partially complementary binding sites, generally located in the 3′ untranslated region (3′ UTR) of target mRNA. Perfect complementarity to a stretch of 7–8 nt at the 5′ end of the miRNA, referred to as seed sequence, is a major determinant in target recognition and is sufficient to trigger gene silencing. The majority of the gene regulatory impact of miRNAs occurs through mRNA degradation, whereas translational repression confers a distinct inhibitory mode of action by miRNAs (3).

MYOMIRS: MICRORNAS IN SKELETAL MUSCLE

Most miRNAs are ubiquitously expressed among tissues. However, a small group of miRNAs is exclusively expressed or enriched in striated muscle, that is, skeletal and cardiac muscle tissue. These muscle-specific miRNAs are referred to as 'myomiRs' and include miR-1, miR-133a, miR-133b, miR-206, miR-208a, miR-208b, miR-486, and miR-499. The tissue specificity of myomiRs results from transcriptional regulation by muscle-specific transcription factors, and is for some myomiRs conferred by their genomic location within the myosin heavy chain (*MyHC*) genes. MyomiRs have been identified as essential determinants in regulatory networks of myogenesis, muscle fiber type composition, muscle growth, and homeostasis, as extensively reviewed by Horak *et al.* [5▪].

Considering the important role of the myomiRs in myogenesis and muscle growth, alterations in their regulation and potential involvement in

muscle atrophy during cachexia can be logically anticipated. Changes in expression of myomiRs have been shown in physiological and disease-induced muscle atrophy, but it is unclear whether these are causally involved or represent adaptive or compensatory responses to muscle atrophy [5▪]. This complexity illustrates the challenge to determine the cause and effect of altered myomiR expression in muscle atrophy. Although a few articles suggested a role for myomiRs in de modulation of catabolic pathways [6], no direct involvement of altered myomiR expression in cachexia has been reported. In contrast, changes in intra-muscular levels of miRNAs not belonging to the myomiRs have been implicated in muscle atrophy and wasting conditions including cachexia.

'ATROMIRS': MICRORNAS INVOLVED IN MUSCLE ATROPHY AND CACHEXIA

Research strategies based on experimental models as well as patient-derived muscle biopsies have been deployed to identify putative miRNAs involved in muscle atrophy and specifically cachexia. We propose to term these miRNAs 'atromiRs'. Using an elegant approach comparing miRNA expression profiles in skeletal muscle of unrelated experimental models of muscle atrophy, Li *et al.* [7▪▪] identified miR-29b as a potential atromiR, as its expression was sufficient and required for the loss of muscle mass. Specifically, a miRNA array was performed on the gastrocnemius muscles from rats subjected to unilateral sciatic denervation-induced muscle atrophy. Of 15 differentially expressed miRNAs, 4 were conserved across species, as their increased expression was confirmed in mouse denervated gastrocnemius muscles. MiR-29b was the only miRNA, which displayed increased expression levels in four additional in vivo models of wasting, including muscle atrophy induced by dexamethasone, fasting, cancer cachexia, and aging (sarcopenia). Furthermore, in fully differentiated myotubes, miR-29b over-expression reduced myotube diameter, and decreased MyHC, indicative of in vitro atrophy [7▪▪]. Additional studies postulate a role for miR-29b in muscle atrophy based on its increased expression in models of cardiac cachexia and aging, and causal involvement has been shown in cell culture experiments, yet its potential involvement in human cachexia awaits confirmation in patient-derived muscle biopsies [7▪▪,8,9].

Comprehensive profiling of miRNAs for cachexia in human muscle biopsies has been scarce, because of challenges posed by heterogeneity of the clinical population, limited group sizes because of the costly profiling techniques and the invasive nature of muscle biopsies collection. In 2017, the first article was published identifying differentially expressed miRNAs between cachectic and non-cachectic cancer patents [10▪▪]. In this study, a total of 8 out of 777 miRNAs from rectus abdominis biopsies from cachectic and non-cachectic pancreas and colorectal cancer patients were found to be differentially expressed. All eight miRNAs were up-regulated, but the direct involvement of the miRNAs reported in this study remains to be addressed in experimental models of cachexia. At the end of 2017, 32 miRNAs were identified to be differentially expressed in the quadriceps of COPD patients with a low fat-free mass index (FFMI) compared with healthy controls [11▪▪]. Most of the differentially expressed miRNAs were down-regulated in the skeletal muscle of patients with COPD. Only six miRNAs were up-regulated with five of them belonging to the same miRNA cluster. This corresponding differential expression suggests their common upstream regulation and putatively coordinated downstream involvement in muscle atrophy. MiR-542-3p and miR-542-5p were the most significantly increased miRNAs in the COPD cohort [11▪▪]. The expression of miR-424-5p, another member of the miRNA cluster, was also highly up-regulated, and inversely proportional to physical performance [12▪]. Remarkably, both the expression of miR-542-5p and miR-424-5p were also found to be significantly increased in patients with intensive care unit-acquired weakness or amyotrophic lateral sclerosis [11▪▪,12▪,13,14]. In both populations, a direct correlation of miR-424−5p expression and disease progression was observed [12▪,13]. These studies are the first to identify differentially expressed miRNAs in patients with cachexia with partial conservation between different diseases [10▪▪,11▪▪]. Importantly, overlapping miRNAs associated with cachexia in cancer as well as COPD, CKD, heart failure, and others, may potentially represent a group of miRNAs specific to muscle atrophy in cachexia per se, independent of the underlying disease (Fig. 1). Therefore, comparative miRNA profiling of biopsies collected from cachectic and non-cachectic patients with these diseases is required for the identification of candidate atromiRs. Subsequently, elucidation of the regulation of atromiR expression and their targets in skeletal muscle will aid to a better understanding of the regulation of muscle breakdown in different wasting conditions.

MICRORNA REGULATION OF PROCESSES AND PATHWAYS THAT GOVERN MUSCLE MASS

Muscle atrophy is the consequence of an imbalance between protein synthesis and degradation.

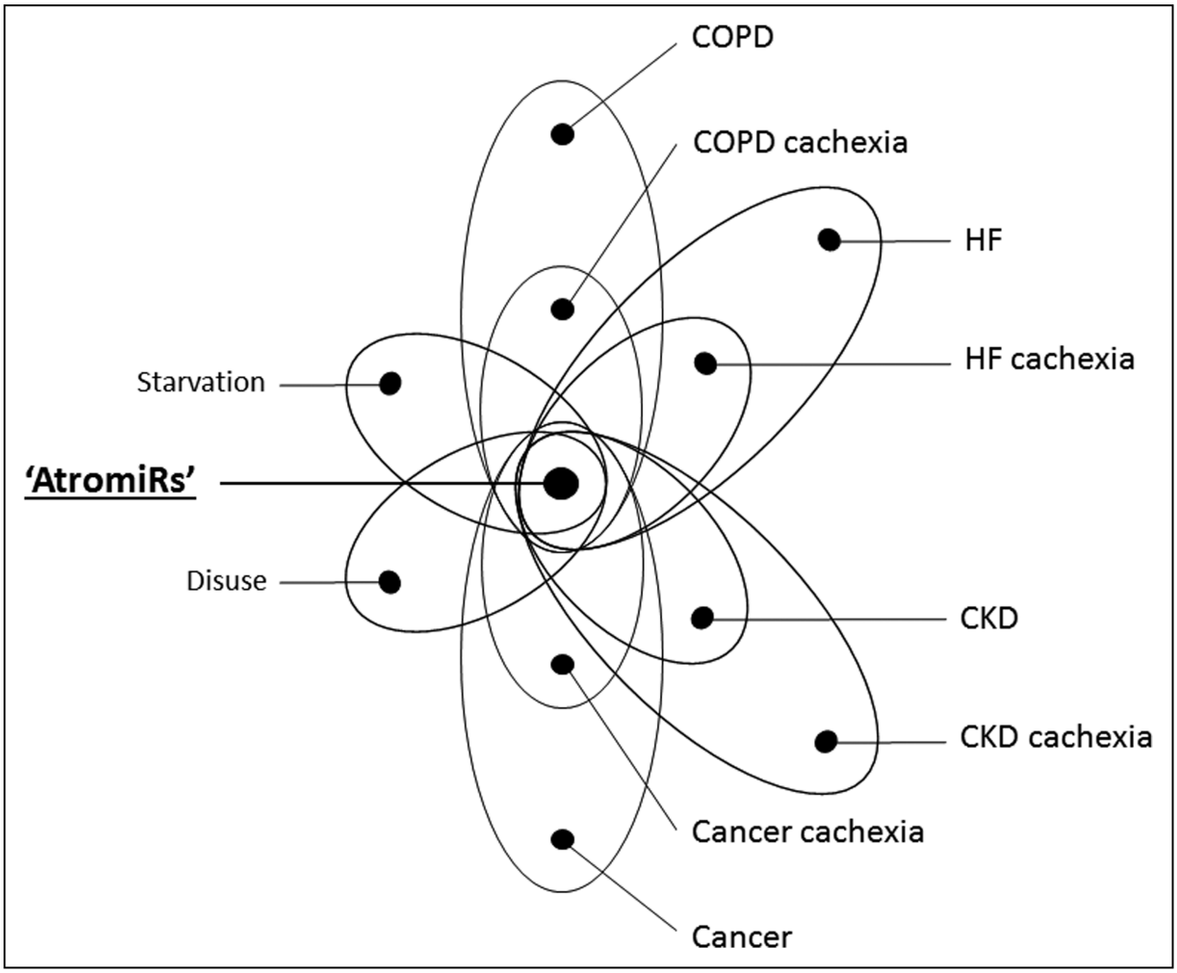

FIGURE 1. Conceptual Venn diagram of differentially expressed miRNAs specifically associated with disease, disease-related muscle atrophy, or muscle atrophy independent of the underlying disease, illustrating the strategy to identify miRNAs in skeletal muscle specifically related to muscle atrophy, 'atromiRs'.

Although the insulin-like growth factor 1 (IGF-1) pathway promotes muscle growth by stimulating protein synthesis and inhibiting proteolysis, signaling induced by ligands of the transforming growth factor beta (TGF-β) family (e.g. myostatin) results in the converse. Specifically, IGF-1 binds the insulin receptor and activates the PI3K-Akt pathway; in turn Akt induces mTOR, which increases mRNA translation efficiency and capacity, although the latter is also determined by the number of ribosomes regulated independently of PI3K/Akt signaling. In contrast, TGF-β receptor activation inhibits Akt through induction of Smad signaling, resulting in repression of protein synthesis. Conversely, the rate of proteolysis depends on the activity of the ubiquitin proteasome pathway (UPP) and the autophagy-lysosome pathway (ALP). Proteolytic activity is in part determined by the expression of rate-limiting enzymes, including the E3 ligase Muscle RING finger 1 (MuRF1) and atrogin-1 in UPP, as well as by components of the ALP. Their expression is in large extent determined by the transcription factors FoxO1/3, which are subject to inhibitory phosphorylation by Akt. Consequently, inverse regulation of Akt activity by the IGF-1 and TGF-β/myostatin pathways defines their opposing effects on proteolysis. In addition, Smad signaling has also been implicated in direct transcriptional regulation of proteolysis [2].

Recently, several miRNAs associated with muscle mass were implicated in the regulation of these processes and pathways (Fig. 2). MiR-424-5p, associated with skeletal muscle loss in different diseases as described above [11▪▪,12▪,13,14], is postulated to modulate protein synthesis. *In vitro*, it was shown that miR-424-5p targets proteins involved in ribosomal RNA (rRNA) synthesis. Over-expression of miR-424-5p in myoblasts reduces both 18S and 47S rRNA. Consistently, miR-424-5p inhibits

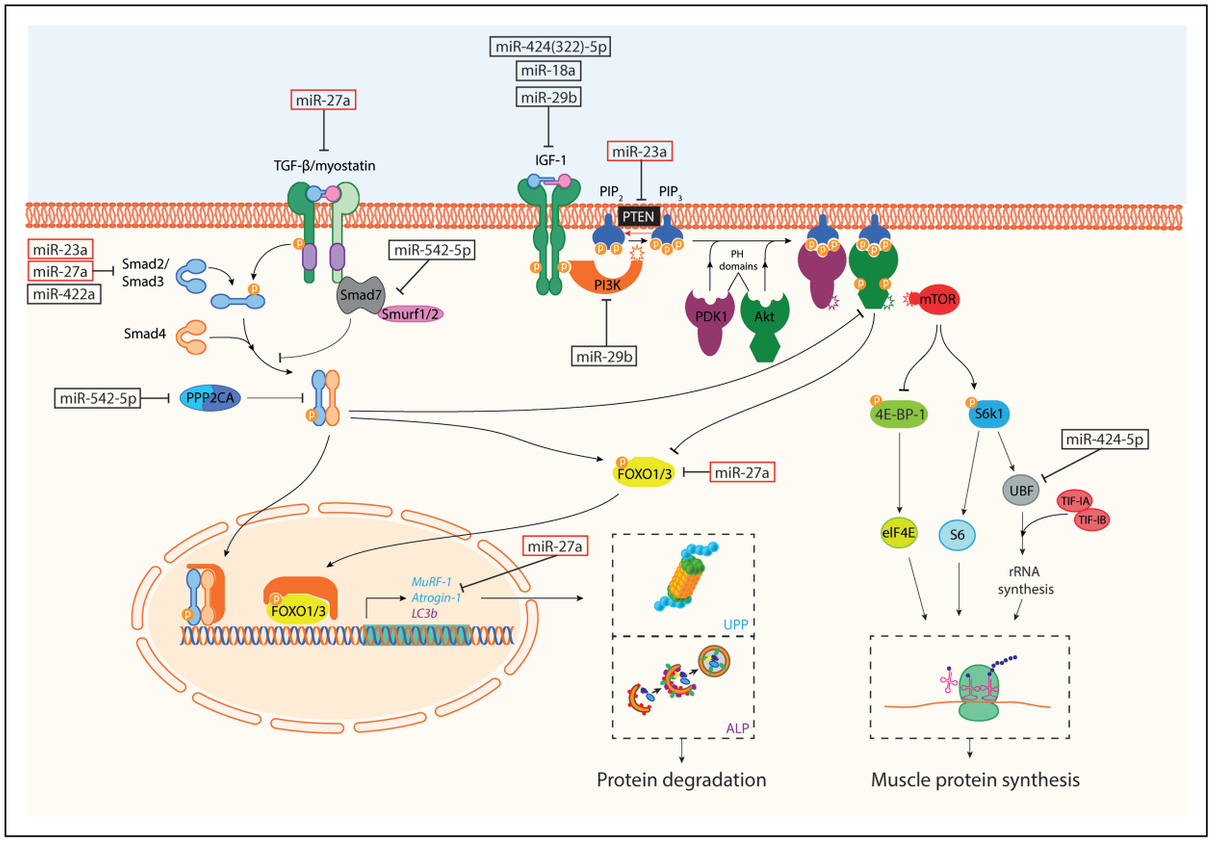

FIGURE 2. Schematic signaling diagram illustrating the potential targets of muscle-derived miRNAs in pathways and processes regulating muscle atrophy. MiRNAs in red boxes correspond to targets only based on in silico target prediction. 4E-BP-1: eukaryotic translation initiation factor 4E-binding protein 1; eIF4E: eukaryotic translation initiation factor 4E; PDK1: phosphoinositide-dependent kinase-1; PIP_2: phosphatidylinositol 4,5-bisphosphate; PIP_3: phosphatidylinositol (3,4,5)-trisphosphate; TIF: transcription initiating factor.

protein synthesis measured by puromycin incorporation. These findings were confirmed *in vivo* by over-expressing miR-322-5p, the rodent orthologue of miR-424-5p, in the tibialis anterior in mice [12▪]. Conversely, expression levels of miR-322-5p are decreased in the hypertrophying right ventricle of rats with pulmonary hypertension [15]. This study also showed that miR-322-5p suppresses IGF-1 expression in C2C12 myoblasts.

Similarly, IGF-1 was also found to be the direct target gene of miR-18a, a miRNA of which expression correlates inversely with muscle growth [16]. *In vitro*, myotube atrophy induced by over-expression of miR-18a was accompanied by increased expression of atrogin-1 and MuRF-1. Furthermore, miR-18a decreased the phosphorylation of both Akt and FoxO3, whereas inhibition of Akt/FoxO signaling blocked these effects of miR-18a [16].

Over-expression of miR-29b, identified as a potential atromiR using experimental models described above [7▪▪], causes myotube atrophy along with elevated expression of MuRF-1 and atrogin-1, and autophagy-related genes. MiR-29b also directly targets IGF-1 and PI3K (p85α), and decreases the phosphorylation levels of downstream proteins such as Akt, FoxO3A, mTOR, and P70S6K. In addition, inhibition of miR-29b expression prevents dexamethasone-induced, TNF-α-induced, and H_2O_2-induced expression of proteolysis-related genes and myotube atrophy [7▪▪].

In contrast to the actions of these miRNAs, the miR-23a/-27a cluster has been shown to attenuate muscle atrophy in a mouse model of CKD-induced muscle wasting. Over-expression of the miR-23a/-27a cluster in the tibialis anterior of CKD mice attenuated muscle loss, improved grip strength and decreased the expression of atrogin-1 and MuRF-1. The latter corresponded with attenuated activity of the PI3k/Akt signaling pathway and reduced Smad2/3 phosphorylation [17]. *In silico*, binding site prediction implicated PTEN and Smad3, and myostatin, Smad2 and FoxO1 as the respective targets of miR-23a and miR-27a potentially responsible for their atrophy-attenuating actions [17,18].

MiR-542-5p has been reported to target inhibitors of the Smad signaling pathway, thereby sensitizing to TGF-β/myostatin ligands [11▪▪]. Smurf1 and Smad7 function as TGF-β type 1 receptor antagonists whereas PPP2CA dephosphorylates the Smad2/3 complex, all suppressing Smad signaling. Over-expression of miR-542-5p in myoblasts reduces the mRNA expression levels of Smurf1, Smad7, and PPP2CA, suggesting that it increases Smad signaling and reduces protein synthesis. These findings were confirmed in mice by overexpressing miR-542-3p in the tibialis anterior [11▪▪]. Conversely, miR-422a was identified as a miRNA positively associated with muscle strength and mass in catabolic diseases. *In vitro*, miR-422a suppressed different members of the Smad family, including Smad2, Smad3 and Smad4, and inhibited TGF-β activity [19▪].

Collectively, multiple studies provide evidence that miRNAs may directly or indirectly regulate protein synthesis and degradation, and upstream signaling pathways indicating their role in muscle atrophy. Further identification of the miRNA targets in these molecular switchboards controlling muscle mass is an essential step when evaluating the potential of miRNA as therapeutic targets for cachexia.

CONCLUSION: MICRORNA THERAPEUTICS FOR CACHEXIA?

Since the discovery of miRNAs, the potential of intervening in miRNA-regulated pathological processes has been investigated and miRNA-based therapeutics have been developed. To date, at least seven miRNA-targeted therapeutics are in clinical trial. The first miRNA-targeted drug to enter phase II clinical trials was Miravirsen, a locked nucleic acid (LNA)-modified antisense inhibitor of miR-122, for the treatment of hepatitis C virus infection [20,21]. Although there are no miRNA therapeutics for muscle atrophy in clinical trial yet, the potential shown in preclinical studies to manipulate miRNA expression and activity through systemic or local delivery raises possibilities for this new class of drugs for cachexia.

A major question in developing miRNA-based therapeutics is whether a single miRNA or miRNA cluster should be targeted. The key obstacle is posed by the numerous mRNA targets engaged by one individual miRNA or cluster. Thus, the manipulation of miRNA expression or function can have a profound impact on cellular homeostasis, making it essential to carefully and comprehensively investigate miRNA expression, and the mRNA targets of a particular miRNA. This should occur not only in skeletal muscle but also in other tissues to ensure off-target effects, for example, on other pathways or in other tissues are limited. In addition, further development of delivery systems with selective or high affinity for skeletal muscle will be required for miRNA-based therapeutics to effectively target skeletal muscle. Finally, the question of whether individual patients express a different set of driver atromiRs remains to be addressed.

The current surge in studies related to miRNAs involvement in cachexia will aid in the identification of key atromiRs for target discovery and drug development. This rapidly increasing body of knowledge, together with comprehensive preclinical research, will clarify the feasibility of miRNA targeting and may eventually bring miRNA therapeutics to the clinic for the treatment of muscle atrophy in cachexia.

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Conflicts of interest

A.V.H. is employed by Nutricia Research.

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- ▪▪ of outstanding interest

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