Skeletal muscle wasting in chronic kidney disease: the emerging role of microRNAs

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Abstract

Skeletal muscle wasting is a common complication of chronic kidney disease (CKD), characterised by the loss of muscle mass, strength and function, which significantly increases the risk of morbidity and mortality in this population. Numerous complications associated with declining renal function and lifestyle activate catabolic pathways and impair muscle regeneration resulting in substantial protein wasting. Evidence suggests that increasing skeletal muscle mass improves outcomes in CKD, making this a clinically important research focus. Despite extensive research, the pathogenesis of skeletal muscle wasting is not completely understood. It is widely recognised that microRNAs (miRNAs), a family of short non-coding RNAs, are pivotal in the regulation of skeletal muscle homeostasis with significant roles in regulating muscle growth, regeneration and metabolism. The abnormal expression of miRNAs in skeletal muscle during disease has been well-described in cellular and animal models of muscle atrophy, and in recent years, the involvement of miRNAs in the regulation of muscle atrophy in CKD has been demonstrated. As this exciting field evolves, there is emerging evidence for the involvement of miRNAs in a beneficial cross-talk system between skeletal muscle and other organs that may potentially limit the progression of CKD. In this article we describe the pathophysiological mechanisms of muscle wasting and explore the contribution of miRNAs to the development of muscle wasting in CKD. We also discuss advances in our understanding of miRNAs in muscle-organ crosstalk and summarise miRNA-based therapeutics currently in clinical trials.

Key words: chronic kidney disease, skeletal muscle wasting, microRNA, satellite cells, protein degradation, cross-talk

Introduction

Chronic kidney disease (CKD) is a global public health concern, with an estimated prevalence of 10-15% of the adult population worldwide (1). In addition to a loss of renal function, CKD is frequently associated with multiple and diverse complications including skeletal muscle wasting (2). Muscle wasting begins early in the disease process, with the rapid loss of muscle mass and strength leading to loss of muscle function, reduced quality of life (3) and increased morbidity and mortality (4).

There are many associated complications of muscle wasting in CKD, including an increased risk of cardio-metabolic disease (5), dysregulation of glucose homeostasis (6), impairments of physical activity (4), and increased risk of hospitalisation (7). The association between skeletal muscle mass, strength, function and mortality, makes this an important clinical consideration in this population. Indeed, interventions which increase muscle mass may improve outcomes for patients with CKD due to the systemic and metabolic effects of skeletal muscle on organs such as the kidney, heart, liver, lungs, and bone (8; 9). Several studies have demonstrated that exercise interventions which increase muscle mass, strength, and function lead to clinically important benefits for patients with CKD (10). These have been extensively reviewed elsewhere (11; 12). Notably, it has been reported that increasing muscle mass independent of exercise provides reno-protective effects in diabetic nephropathy (13), obstructive kidney disease (14), and acute kidney injury (15), thereby presenting a possible therapeutic avenue for mitigating disease progression in such diseases.

Interventions that prevent or attenuate muscle atrophy whilst providing protection against the progression of CKD are highly desirable, but at present, such therapeutic interventions are not in routine clinical practice. To develop strategies with potential to improve skeletal muscle mass, strength, and function, the key pathophysiological processes driving this complication need defining more completely. microRNAs (miRNAs) are increasingly recognised as a key part of this process, and multiple studies have independently identified the major contribution of aberrant miRNA expression and regulation to the development of muscle atrophy in both primary (16) and secondary (17) muscle wasting conditions.

This review will give an overview of the mechanisms of muscle wasting in CKD and summarise the current literature with regard to miRNAs and their role in CKD-associated skeletal muscle wasting.

Mechanisms of muscle wasting

Previously, malnutrition was thought to stimulate protein loss in patients with CKD, however, increasing dietary protein does not eliminate protein loss and experimental models of CKD have confirmed the involvement of more complex cellular mechanisms (18). Muscle mass is maintained by the balance of protein metabolism, and small but persistent imbalances between protein synthesis and degradation will induce muscle wasting. It is now recognised that the catabolic environment of CKD, which includes metabolic acidosis, inflammation, increased glucocorticoid production and suppressed insulin/IGF-1 signalling, stimulates and accelerates substantial muscle protein loss through the activation of protein degradation, suppression of protein synthesis, and impairment of muscle regeneration (19).

Altered muscle protein metabolism

The IGF-1-Akt-mTOR signalling pathway is critical in the regulation of skeletal muscle mass, being the major determinant of skeletal muscle growth or atrophy (20). It is widely accepted that this pathway is disturbed in CKD, with the suppression of anabolic factors, such as insulin/IGF1, leading to decreased Akt-mTOR signalling, which has a dual effect.

Firstly, decreased insulin/IGF-1 signalling promotes muscle protein degradation via a reduction in levels of downstream phosphorylated-Akt (p-Akt). This triggers the Forkhead box O (FoxO) transcription factor-mediated induction of two muscle-specific E3 ubiquitin ligases or 'atrogenes', F-box only protein 32 (MAFbx, also known as atrogin-1) and Tripartite Motif Containing 63 (TRIM63, also known as MuRF-1), which stimulate muscle proteolysis by catalysing the ubiquitination and degradation of key muscle proteins via the ubiquitin proteasome system (UPS; Figure 1) (21; 22). It has been demonstrated that activation of FoxO by itself can cause profound muscle atrophy, and inhibition of FoxO function reduced the induction of atrogin-1 and MuRF-1 (21; 23). Moreover, mice deficient in either atrogin-1 or MuRF-1 were found to be resistant to atrophy altogether (23). In CKD, FoxO1, FoxO3, atrogin-1 and MuRF-1 mRNA levels are increased in atrophying muscles signifying increased protein degradation (24). Many studies also confirm the major role of the UPS in muscle proteolysis, and the expression of genes encoding its components are increased in CKD (25). Furthermore, myostatin, a critical negative regulator of skeletal muscle mass (26), is up-regulated in conditions that cause muscle wasting. Myostatin is predominantly expressed in skeletal muscle, and functions by binding to its cell surface receptor, activin A receptor type IIB (ActRIIB), leading to the phosphorylation and subsequent activation of transcription factors Smad2 and Smad3, which further recruit Smad4, forming a heterodimeric Smad2-4 complex (Figure 1) (27). This complex is the key

intracellular mediator for myostatin as it translocates to the nucleus and directly stimulates atrogenic transcription and protein wasting. Additionally, the phosphorylation of Smad2/Smad3 results in a further reduction of p-Akt, which again perpetuates the cycle of muscle protein degradation (27). The inhibition of myostatin prevents muscle atrophy in CKD by suppressing protein degradation, thus confirming its key involvement in muscle wasting (28).

Secondly, the uremic state significantly reduces protein synthesis via impaired basal signalling through the mTOR anabolic pathway (29). A reduction in the level of basal p-Akt has been reported in the skeletal muscle of both animal models of CKD and patients with end-stage renal disease (30; 31). Decreased levels of p-Akt reduces the activation of downstream kinases, serine/threonine-protein kinase mTOR and ribosomal protein S6 kinase (p70S6K; Figure 1). This significantly reduces the synthesis rate of mixed muscle proteins, myosin heavy chains (MyHC), and muscle mitochondrial oxidative enzymes (27%, 37% and 27% reductions, respectively) (29).

Together, increased protein degradation and decreased protein synthesis lead to a dramatic decrease in muscle cell protein and myofibre size. However, evidence strongly suggests that the persistent activation of muscle protein breakdown is the primary cause of wasting in CKD (19).

Impaired muscle regeneration

Muscle wasting may also result from the impaired capacity of muscle fibres to regenerate. Skeletal muscle is a post-mitotic tissue containing terminally differentiated cells. Its ability to regenerate is dependent upon the presence of muscle stem cells, termed satellite cells, responsible for the growth, maintenance, and repair of skeletal muscle (32). Although the evidence base is limited, there are data suggesting that CKD compromises satellite cell function, reducing their regenerative capacity, and contributing to the process of muscle wasting (33).

Satellite cells are usually quiescent, becoming activated in response to injury or exercise, or to changes in levels of growth factors, such as IGF-1. Once activated, they proliferate and differentiate into myofibrils, which can fuse to form mature muscle fibres in a process known at a cellular level as myogenesis, which precedes muscle regeneration (32). Myogenesis is regulated by serum response factor and myocyte enhancer factor-2, in combination with myogenic regulatory factors (MRFs), including Pax7, Myf5, MyoD, MRF4, and myogenin. Quiescent satellite cells constitutively express Pax7 and upon activation Myf5 and MyoD upregulation triggers their proliferation as myoblasts. Myoblasts are stimulated to downregulate Pax7 and express MRF4 and myogenin, at which point

they are committed to terminally differentiate. Immature myofibrils express embryonic MyHC (eMyHC; a marker of differentiation and fusion), exit the cell cycle and fuse to form mature multinucleated myofibres, which repair or enlarge muscle fibres. In a mouse model of CKD, reduced levels of MRFs and eMyHC suppressed satellite cell activation and differentiation, significantly impairing myogenesis, and hence muscle regeneration *in vivo* (34). Satellite cell dysfunction and reduced MRF expression in CKD has also been linked to both decreased IGF-1, and increased in myostatin (30; 19).

The role of miRNAs in skeletal muscle

While the mechanisms of muscle atrophy have been extensively studied, the key processes driving this complication in CKD need defining more completely. One potential mechanism is the aberrant expression of a family of short (18-25 nucleotides) non-coding RNA molecules, known as miRNAs, which have emerged as key negative regulators of gene expression. They are implicated in nearly every biological pathway, fine-tuning gene expression through a post-transcriptional mechanism that induces either translational repression or degradation of target messenger RNAs (mRNAs). miRNAs identify their targets based on sequence complementarity between the 3' untranslated region of the target mRNA and their 'seed sequence', which is located between nucleotides 2 and 7 of the miRNA (35). Several miRNAs can simultaneously regulate the expression of a single protein, whilst a single miRNA can target a number of different proteins, providing a layer of complexity to the regulation of gene expression (35). Through this mechanism, miRNAs can control the diversity of proteins within a cell, and consequently cellular activity. It is therefore unsurprising that dysregulated miRNA expression has been implicated in many diverse pathologies.

miRNAs are critical in the maintenance of skeletal muscle homeostasis, with significant roles in the regulation of muscle growth, regeneration and metabolism. They can be sub-divided into two categories: miRNAs exclusively expressed in muscle, termed 'myomiRs', and those expressed broadly across many cell types. MyomiRs, namely miRNA-1, miRNA-133, and miRNA-206, are important regulators of skeletal muscle homeostasis with crucial roles in muscle metabolism and myogenesis. Besides myomiRs, many non-muscle-specific miRNAs play key roles in the regulation of skeletal muscle homeostasis, including miRNA-31 (36), miRNA-29 (37), and miRNA-486 (38). These miRNAs have been implicated in the regulation of myogenesis, with significant roles in satellite cell activation and differentiation, and in the regulation of insulin signalling and the myostatin cascade.

Below we outline the involvement of miRNAs in the regulation of muscle protein metabolism and muscle regeneration and discuss the current understanding of how these pathways are affected in CKD to cause muscle wasting.

miRNAs and muscle protein metabolism

Numerous studies demonstrate that aberrant skeletal muscle miRNA expression stimulates skeletal muscle atrophy through abnormal regulation of protein metabolism in catabolic conditions (Table 1; Figure 1). For example, miRNA-486 targets phosphatase and tensin homolog (PTEN) and FoxO 1a to increase levels of p-Akt and down-regulate muscle proteolysis. However, during catabolic conditions myostatin stimulates repression of the miRNA-486/ Ankyrin 1.5 promoter, leading to decreased

miRNA-486 expression, reduced p-Akt and increased protein degradation *in vitro* (39). Similarly, the inflammatory cytokine TNF-like weak inducer of apoptosis (TWEAK), a potent inducer of skeletal muscle wasting, inhibits the expression of several miRNAs including muscle-specific miRNA-1-1, miRNA-1-2, miRNA-133a, miRNA-133b, and miRNA-206, as well as significantly increasing the expression of miRNA-146a and miRNA-455 in TWEAK-treated myotubes (40). These miRNAs target mRNAs involved in proteolytic degradation, the inflammatory response, fibrosis and extracellular matrix remodelling (40), indicating miRNAs might be responsible for mediating TWEAK-induced skeletal muscle loss in various chronic diseases.

These data confirm a role for miRNAs in mediating the effects of catabolic factors to activate protein degradation. Notably, experimental manipulation of miRNA expression can reverse or even protect against skeletal muscle atrophy during disease, signifying the substantial contribution of miRNAs to the regulation of skeletal muscle mass and function in muscle wasting (Table 1).

miRNAs and muscle regeneration

There has been significant advancement in our understanding of the central role played by miRNAs in the regulation of satellite cell function. miRNAs are critical in both the maintenance of satellite cell pluripotency (41), and in the rapid transition of satellite cells from proliferation to differentiation by targeting important regulators of myogenesis (Table 1). miRNA transcriptome profiling has identified 351 miRNAs differentially regulated in quiescent versus activated satellite cells (42), and a separate study identified 60 miRNAs that are differentially expressed during myogenic proliferation and differentiation (43). For example, the transcriptional activation of three pairs of bicistronic myomiRs, miRNA-1-1 and miR-133a-2, miR-1-2 and miR-133a-1, and miR-206 and miR-133b, by the MRFs regulates muscle gene expression during myogenesis (44).

Unsurprisingly, miRNA dysregulation has been implicated in the impairment of myogenesis, and hence muscle regeneration during disease. Meyer et al. investigated how TNF- α regulates miRNA expression to affect myoblast differentiation and myotube formation (45). Their findings indicate that miRNAs mediate the inhibitory effects of TNF- α on myoblast differentiation by affecting the fusion capacity of myoblasts through targeting genes associated with axon guidance, focal adhesion and neurotrophin signalling. They demonstrated that inhibition of miRNA-155 combined with overexpression of miRNA-503 promotes myotube formation, opposing TNF- α 's inhibitory effects. Conversely, Motohashi et al. (46) suggest that TNF- α promotes myotube maturation and myofibre hypertrophy via the negative regulation of miRNA-128a, which facilitates insulin signalling. In

another study, Winbanks et al. (47) showed that miRNA-206 and miRNA-29 are downregulated by TGF- β , which leads to an up-regulation of histone deacetylase 4 (HDAC4), ultimately triggering the inhibition of myogenic differentiation. The authors demonstrated that overexpression of miRNA-206 and miRNA-29 target HDAC4 to attenuate the inhibitory actions of TGF- β on satellite cell differentiation.

These data highlight the advancement in our understanding of the central role played by miRNAs in the mechanisms that control satellite cell function, as well as the consequences of their dysregulation for muscle regeneration.

miRNAs and CKD-associated skeletal muscle wasting

To date, few studies have specifically investigated CKD-associated muscle wasting and miRNAs. One study examined a profile of miRNAs in skeletal muscle from mice with CKD and sham-operated, pair-fed controls (37), observing the differential expression of 12 miRNAs (Table 2). The investigators focused on the significantly decreased expression of miRNA-29, reporting that increased levels of inflammatory cytokines in CKD activate NF- κ B signalling, inhibit miRNA-29 transcription and reduce the differentiation of myoblasts through upregulation of its target Yin Yang-1 (YY1). YY1 inhibits the synthesis of late-stage, differentiation genes, which was confirmed experimentally when satellite cells isolated from a mouse-model of CKD developed fewer myotubes and exhibited reduced maturation when compared to controls. Upon miRNA-29 overexpression, YY1 was suppressed and differentiation was substantially improved in myoblasts from CKD mice. As a result of these experimental findings, the authors proposed a mechanism for involvement of miRNAs in the impairment of muscle regeneration in CKD.

In another study, Xu and colleagues showed that miRNA-486 was significantly decreased in the skeletal muscle of CKD mice (38). The authors hypothesised that enhancing miRNA-486 expression might block muscle wasting through targeting of PTEN and FoxO1 and they explored this hypothesis in two distinct models of muscle wasting: one model of CKD mice and another mouse administered with a high dose of glucocorticoids. They demonstrated that *in vivo* electroporation of miRNA-486 into muscles can protect against CKD-induced skeletal muscle wasting and increase muscle mass despite CKD. The overexpression of miRNA-486 targets PTEN and FoxO1 to enhance Akt-signalling and block atrogen-1 and MuRF-1 upregulation, thus muscle proteolysis in mice with CKD. These observations in combination with miRNA-486's role in myogenesis highlight the importance of this miRNA in regulating multiple facets of the atrophy programme (48).

These studies demonstrate the important role of miRNAs in the regulation of muscle atrophy in CKD. Knowledge of these pathways might enable the development of miRNA-based therapeutics for the treatment of muscle wasting in CKD and other catabolic conditions.

miRNAs and exercise in CKD

There have been some reports of success in improving muscle atrophy in patients with varying severities of CKD using interventions such as exercise to improve muscle mass and strength, cardiorespiratory fitness, and quality of life (12). Exercise enhances IGF-1-Akt-mTOR signalling in both CKD mice and patients to attenuate muscle loss (10). The role of miRNAs in mediating the beneficial effects of exercise through targeting key proteins in this pathway is beginning to be defined. A study exploring the use of low frequency electrical stimulation (LFES) to mimic resistance exercise reported the attenuation of CKD-induced skeletal muscle atrophy in mice through altered myomiR expression (49). LFES caused an initial decrease in the expression of miRNA-1 and miRNA-206, which increased IGF-1 levels and suppressed protein degradation through upregulation of IGF-1 signalling. This transient reduction in miRNA-1 and miRNA-206 during LFES also correlates well with active satellite cell proliferation during the early phase of muscle regeneration (50). In addition to this, at a later phase after LFES, miRNA-1 and miRNA-206 expression increased and remained elevated in muscle over a sustained period, which promoted myogenesis by targeting Pax7. This significantly reversed the CKD-induced suppression of MRFs, including MyoD and myogenin, and eMyHC. Thus, LFES improved both muscle protein metabolic status and muscle regeneration capacity, however, the authors concluded that the effect of LFES on myomiRs needs additional study to further elucidate the mechanisms driving their altered expression.

Resistance training has also been reported to significantly increase the levels of miRNA-23a and -27a in skeletal muscle of CKD mice, and improve muscle atrophy in this model (51). Notably, overexpression of an adeno-associated virus-miRNA-23a/27a/24-2 cluster precursor in the tibialis anterior (TA) muscle of mice reversed many of the catabolic responses induced by CKD in the absence of exercise. The cluster restored Akt-signalling, decreased PTEN and FoxO1 activation, as well as improved markers of muscle regeneration, including increased levels of MyoD, myogenin and eMyHC. It also opposed the CKD-induced increase in myostatin, thereby reducing signal transduction via p-SMAD2/3. Indeed, these responses translated into greater muscle mass and strength despite CKD, as measured by grip strength (51). These results suggest that miRNA-23a and -27a play a critical role in the regulation of muscle mass, and when considered together, outline the potential of miRNAs to be developed as a therapeutic tool, which could provide an alternative to exercise for increasing muscle mass in CKD and other muscle wasting conditions. Whilst the additional benefits associated with exercise would need to be carefully considered here, this is an attractive option in CKD patients who are often elderly and unable to undertake regular intense physical activity due to their frailty (52).

A role for miRNAs in muscle-organ crosstalk?

Recent research investigates the possibility that interactions, termed crosstalk, between skeletal muscle and other organs can limit the progression of kidney disease. One mechanism of interaction may involve the release of exosomes in the muscle 'secretome', which facilitate communication between cells. Exosomes are small (30-120nm) membrane vesicles of endocytic origin released into the extracellular environment (53). Hudson et al. demonstrated for the first time that skeletal muscle selectively packages miRNAs into exosomes during conditions of atrophy (54). In this study, glucocorticoid administration significantly reduced the levels of miRNA-23a in C2C12 myotubes through rapid incorporation into exosomes that were released from myotubes. This presents a novel mechanism by which skeletal muscle signals to other tissues during muscle atrophy. Recent evidence has implicated miRNAs in crosstalk between skeletal muscle and the kidneys through this mechanism. Investigators found that overexpression of the miRNA-23a/27a cluster in the TA of STZ-induced diabetic mice significantly attenuated muscle loss, improved muscle function and reduced renal fibrosis (13). The authors examined the possibility that miR-23a/27a produced in the muscle of STZ-induced diabetic mice could be delivered via exosomes to the kidney where they influence the processes of renal fibrosis. Consistent with a crosstalk model, levels of mature miRNA-23a/27a were significantly increased in serum exosomes and kidneys compared to control virus-injected mice (13). In another study, injection of exosome-encapsulated miRNA-29 (Exo/miR-29) in the TA of unilateral ureteral obstruction mice counteracted both muscle atrophy and renal fibrosis (55). The authors confirmed that following provision of Exo/miR-29 to the TA, miRNA-29 was increased in circulating exosomes and in the kidney where it inhibits several fibrosis-related proteins, including YY1 and TGF- β 3. These studies provide early evidence to support the existence of a beneficial muscle-kidney crosstalk system that is mediated by miRNAs released in the muscle 'secretome', which ameliorate renal fibrosis lesions and provide a reno-protective effect in mice at risk of progressive kidney disease.

The role of miRNAs in mediating crosstalk between skeletal muscle and the heart has also been investigated (56). Deep sequencing analysis revealed decreased levels of miRNA-26a in cardiac and skeletal muscle of CKD versus sham-operated pair-fed mice, which the authors found to be associated with skeletal muscle atrophy and cardiomyopathy in CKD. Investigators demonstrated that injection of exosome-encapsulated miRNA-26a (Exo-miR-26a) in the TA muscle of CKD mice significantly attenuated CKD-induced skeletal muscle wasting, and decreased evidence of cardiomyopathy in this model. Provision of miRNA-26a to the TA muscle of CKD mice significantly reduced indicators of uremic cardiomyopathy, through targeting of multiple proteins involved in insulin resistance and fibrosis, and improved parameters of cardiac function despite CKD. Consistent

with muscle-heart crosstalk, intramuscular injection of Exo-miR-26a increased the levels of miRNA-26a in serum exosomes, contralateral un-injected TA muscle and in the heart. Notably, miRNA-26a expression was significantly higher in uremic hearts compared to sham-control hearts following injection of Exo-miR-26a, which led investigators to hypothesise that injured organs have a higher capacity to recruit exosomes containing miRNAs than do uninjured organs. This merits further investigation as it could allow for the development of targeted miRNA therapies to injured organs during disease.

These findings provide new insights into the role of miRNAs in mediating a beneficial muscle-organ crosstalk system and should be considered in the development of new strategies to simultaneously treat the multi-morbidity that commonly associates with kidney disease.

miRNAs in the clinic

The role of miRNAs as key mediators of pathologic mechanisms has led to the development of miRNA-based therapeutics for the treatment of several diseases (reviewed in (57)). There are two distinct classes of miRNA-based therapeutics: miRNA mimics, which aim to replenish miRNA expression, and miRNA inhibitors (also known as antimiRs or antagomiRs), which inhibit a selected miRNA by binding to it strongly. To date, miRNA-based therapeutics have shown great promise in pre-clinical studies, and several have successfully completed, or are ongoing in phase I and II clinical trials (Table 3). Although some challenges remain, the targeting of miRNA function is an attractive and viable option for the treatment of many aspects of human disease. As this exciting field progresses, miRNA-based therapeutics will undoubtedly become candidates for the treatment of skeletal muscle wasting in CKD and other metabolic diseases.

Conclusions

In summary, there has been significant advancement in our understanding of the central role of miRNAs in regulating skeletal muscle homeostasis. The consequence of aberrant miRNA expression on skeletal muscle mass and function is now well defined in several diseases. Whilst a limited number of studies specifically investigate the role of miRNAs in CKD muscle wasting, research strongly suggests that miRNAs are pivotal to the abnormal modulation of protein metabolism and satellite cell function seen in this population. Furthermore, preliminary evidence implicates miRNAs in a beneficial muscle-organ crosstalk system that results from increasing skeletal muscle mass in CKD. This phenomenon holds great potential for the development of strategies that simultaneously treat the multiple complications of kidney disease. Current knowledge about the roles of miRNAs in muscle wasting and cross-talk is restricted to animal models of CKD, and therefore, future research should focus on identifying miRNAs central to muscle wasting and muscle-organ crosstalk in human CKD. The recent successes of several miRNA-based drugs in phase I and II clinical trials hold great promise for the development of miRNA-based therapeutics that target multiple signalling pathways to potentially increase skeletal muscle mass and limit the progression of CKD.

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Conflict of interest statement

None of the authors have any conflicts to declare, I can confirm the results presented in this paper have not been published previously in whole or part.

Tables

Table 1. Summary of key studies of microRNAs and skeletal muscle

miRNAs and protein metabolism				
miRNA	Target	Action	Expression in catabolic conditions	Model
miRNA-182 (58)	FoxO3	Reduced expression in response to dexamethasone, which stimulates atrophy Overexpression blocks dexamethasone-induced increase in FoxO3 to ameliorate protein degradation	↓	C2C12 mouse myoblast cell line
miRNA-23a (55; 59; 51)	atrogin-1, MuRF-1, PTEN and caspase-7	Reduced expression in response to glucocorticoid administration via a mechanism that includes its selective packaging into exosomes for release Resistance exercise increases expression in CKD mice to maintain muscle mass Overexpression attenuates muscle loss <i>in vitro</i> and <i>in vivo</i> miRNA-23a transgenic mice display resistance to glucocorticoid-induced muscle wasting	↓ in CKD	C57BL/6 mice CKD mice (two-stage 5/6 nephrectomy) C2C12 mouse myoblast cell line
miRNA-1 (60)	HSP70	Mediates atrophy in response to high doses of myostatin and Dexamethasone Reduces HSP70 levels, resulting in decreased p-Akt and activation of FoxO3 target proteins, atrogin-1 and MuRF-1 Inhibition attenuates the Dexamethasone-induced increase in atrophy-related proteins	↑	C57BL/6J mice C2C12 mouse myoblast cell line
miRNA-486 (38)	PTEN and FoxO1a	miRNA-486 co-ordinately decreases FoxO1 and PTEN to protect against muscle atrophy	↓ in CKD	MFKO mice with CKD (two-stage nephrectomy)

		Overexpression protects against dexamethasone-induced muscle wasting <i>in vitro</i> and increases muscle mass despite CKD <i>in vivo</i>		Primary satellite cells from mouse model
miRNA-18a (61)	IGF-1	Expression down-regulated during myoblast differentiation	Not determined	C57BL/6 mice
		Overexpression increases the expression of MuRF-1 and atrogin-1 and induces myotubes atrophy		C2C12 mouse myoblast cell line
		Inhibition during fasting-induced atrophy promotes the expression of IGF-1 and protein synthesis, hence protecting against muscle atrophy		
miRNA-26a (56)	FoxO1, PTEN and GSK-3 β	Decreased expression in CKD, which stimulates muscle atrophy	↓ in CKD	5/6 nephrectomised CKD mice
		Overexpression of miRNA-26a in muscle prevented CKD-induced muscle wasting <i>in vivo</i>		
miRNA-424 (62)	UBTF, PolR1A, and RRN3	Increased expression in conditions of muscle wasting	↑	C2C12 mouse and LHCN-M2 human myoblast cell line
		Regulates rRNA synthesis by inhibiting Pol I pre-initiation complex formation, which reduces the capacity of protein synthesis machinery, contributing to the inhibition of protein synthesis and loss of muscle mass		C57BL/6 mice
		Overexpression <i>in vivo</i> causes a marked reduction in muscle size		Skeletal muscle biopsy from COPD, intensive care unit-acquired wasting or aortic surgery patients
miRNAs and satellite cell function				
miRNA	Target	Action	Stage/Expression during myogenesis	Model
miRNA-489 (42)	DEK	Highly enriched in satellite cells, essential for the maintenance of quiescence	Quiescence/↓	Primary satellite cells from mice
		Prevents proliferative expansion during quiescence through targeting of DEK, rapidly downregulated upon satellite cell activation		
		Sustained expression results in impaired muscle regeneration <i>in vivo</i>		

miRNA-31 (36)	Myf5	Inhibition causes satellite cells to spontaneously enter the cell cycle	Quiescence/↓	Primary satellite cells from mice
		Holds satellite cells poised to enter myogenesis through sequestration of Myf5 mRNA in mRNP granules during quiescence		
		Upon activation, Myf5 mRNA re-enters translation, which promotes myogenesis		
miRNA-17, miRNA-20a and miRNA-92a (63)	ENH1	Manipulation of miRNA-31 levels affects satellite cells differentiation <i>ex vivo</i> and muscle regeneration <i>in vivo</i>	Proliferation/↓	C2C12 mouse myoblast cell line C57BL/6 mice
		Represses myogenic differentiation by targeting actin-associated protein ENH1, which increases nuclear accumulation of ID1 (inhibitor of differentiation 1) to block MRFs		
		Dramatically downregulated after myogenesis stimulation		
miRNA-17 (64)	Ccnd2, Jak1, Rhoc	Overexpression delays muscle regeneration <i>in vivo</i>	Differentiation/not determined	C2C12 mouse myoblast cell line C57BL/6 mice
		Potent inducer of skeletal muscle differentiation		
		Inhibition of proliferation by miRNA-17 induces apoptosis, which limits efficacy of miRNA-17 to induce myogenesis		
miRNA-199a (65)	IGF-1, mTOR, RPS6KA6	Apoptosis is partially reversed upon addition of miRNA-19 to facilitate myotube maturation	Differentiation/↑	C2C12 mouse myoblast cell line
		Increased miRNA-17 and miRNA-19 expression in tibialis anterior muscles <i>in vivo</i> advanced muscle regeneration		
		Regulates myogenesis through suppression of the IGF-1/Akt/mTOR signalling pathway		
		Overexpression partially blocks differentiation and activation of Akt/mTOR signalling pathway		
		Inhibition promotes differentiation and myotube hypertrophy		

miRNA-1 (66; 67)	Pax7, HDAC4	Sharply upregulated during satellite cell differentiation to restrict proliferation	Differentiation/↑	Bovine skeletal muscle primary satellite cells
		Alleviates the repression MEF2 by targeting HDAC4, which promotes differentiation		Mouse skeletal muscle primary satellite cells
		Inhibition significantly delays satellite cell differentiation and enhances proliferation		C2C12 mouse myoblast cell line
miRNA-206 (47; 48; 68)	Pax7, HDAC4, p180 subunit of DNA polymerase-α	Induced during differentiation to restrict proliferation	Differentiation/↑	C2C12 mouse myoblast cell line
		p180 subunit of DNA polymerase α inhibits DNA synthesis, an important component of the differentiation programme		
		Inhibition blocks cell cycle withdrawal and significantly delays satellite cell differentiation partly due to persistent Pax7 expression, which drives the expression of Id2, an inhibitor of MyoD		
miRNA-486 (48)	Pax7	Upregulated with miRNA-206 during satellite cell differentiation	Differentiation/↑	C2C12 mouse myoblast cell line
		Together with miRNA-206 acts as a bi-stable switch to regulate a Pax7-driven myoblast state or a MyoD-driven myotube state		

Table 2. microRNAs significantly increased or decreased in muscles of CKD versus sham-operated pair-fed control mice (37)

<i>miRNA</i>	<i>Expression levels (fold-change)</i>
hsa-miR-181d	Increased (5.08)
mmu-miR434-5p	Increased (6.624)
mmu-miR-455-3p	Increased (2.848)
mmu-miR-496	Increased (3.669)
mmu-668	Increased (4.088)
hsa-miR-23a	Decreased (-3.726)
hsa-miR-29a	Decreased (-4.181)
hsa-miR-29b	Decreased (-3.732)
mmu-miR-124a	Decreased (-4.181)
mmu-miR-187	Decreased (-43.93)
mmu-miR-199b	Decreased (-3.732)
hsa-miR-376a	Decreased (-10.204)

Table 3. miRNA-based therapeutics in clinical trials

Name (sponsor)	Therapeutic agent	Disease	Phase	clinicaltrials.gov identifier
MesomiR 1 (EnGeneIC Limited)	miRNA-16 mimic	Malignant Pleural Mesothelioma and Non-Small Cell Lung Cancer	Phase I, completed	NCT02369198
MRG-201 (miRagen Therapeutics, Inc)	miRNA-29b mimic	Pathologic fibrosis	Phase I, completed	NCT02603224
			Phase II, active not recruiting	NCT03601052
MRX34 (Mirna Therapeutics, Inc)	miRNA-34a mimic	Multiple solid tumors	Phase I, terminated	NCT01829971
			Phase II, withdrawn	NCT02862145
Miravirsen (Santaris Pharma A/S and Hoffmann-La Roche)	miRNA-122 inhibitor	Hepatitis C, Chronic Hepatitis C	Phase I, completed	NCT01646489
			Phase II, completed	NCT01200420
			Phase II, unknown	NCT01872936
			Phase II, unknown	NCT01727934
			Phase II extension, completed	NCT02508090
			Phase II extension, completed	NCT02452814
MRG-110 (miRagen Therapeutics, Inc)	miRNA-92a inhibitor	Ischemic conditions	Phase I, completed	NCT03603431

MRG-106 (miRagen Therapeutics, Inc)	miRNA-155 inhibitor	Blood cancers	Phase I, recruiting	NCT02580552
			Phase II, recruiting	NCT03713320
			Phase II, not yet recruiting	NCT03837457
microRNAs and microRNA inhibitors (Gustavo Avila-Ortiz)	miRNA-200a inhibitor and miRNA-200c mimic	Tooth extraction	Phase I, not yet recruiting	NCT02579187

Figure Legend

Figure 1. Interaction between microRNAs and intracellular signalling pathways regulating muscle wasting in catabolic conditions. In CKD expression of miRNA-486 (targets FoxO1 and PTEN) and miRNA-23a (targets PTEN, MuRF-1 and atrogin-1) is significantly decreased, which leads to increased MuRF-1 and atrogin-1, resulting in increased protein degradation. In catabolic conditions, expression of miRNA-182 (targets FoxO3) is decreased, which results in increased activation of atrogin-1 and MuRF-1 and protein degradation. During fasting-induced atrophy, overexpression of miRNA-18a (targets IGF-1) results in decreased IGF-1 signalling and increased protein degradation. In catabolic conditions, expression of miRNA-1 (targets HSP70, which binds to and protects p-Akt) is increased, which indirectly decreases p-Akt leading to increased protein degradation. Colours denote; orange, ligand; white, receptor; purple, intracellular mediator; red, microRNA (miRNA); green, nucleus; arrows indicate direction of expression and/or activity in muscle atrophy.