Role of microRNA in muscle regeneration and diseases related to muscle dysfunction in atrophy, cachexia, osteoporosis, and osteoarthritis

MicroRNAs (miRNAs) are a class of small non-coding RNAs that have emerged as potential predictive, prognostic, and therapeutic biomarkers, relevant to many pathophysiological conditions including limb immobilization, osteoarthritis, sarcopenia, and cachexia. Impaired musculoskeletal homeostasis leads to distinct muscle atrophies. Understanding miRNA involvement in the molecular mechanisms underpinning conditions such as muscle wasting may be critical to developing new strategies to improve patient management. MicroRNAs are powerful post-transcriptional regulators of gene expression in muscle and, importantly, are also detectable in the circulation. MicroRNAs are established modulators of muscle satellite stem cell activation, proliferation, and differentiation, however, there have been limited human studies that investigate miRNAs in muscle wasting. This narrative review summarizes the current knowledge as to the role of miRNAs in the skeletal muscle differentiation and atrophy, synthesizing the findings of published data.

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Article focus
- Accumulating evidence suggests a significant role of microRNA (miRNA) in muscle differentiation and an important role of miRNAs as regulators of muscle atrophy.
- This review summarizes the role of miRNAs in skeletal muscle differentiation and atrophy and offers future research directions.
- MicroRNAs are involved in muscle atrophies associated with different pathophysiological conditions.

Key messages
- MicroRNAs regulate myogenesis, through the control of satellite cell quiescence, proliferation, and terminal differentiation.
- There is no consensus on how miRNA dysregulation alters the pattern of muscle wasting related to different pathophysologies.
- More research is required to understand the involvement of miRNAs in muscle atrophy pathways before these targets can be used as clinical biomarkers.

Strengths and limitations
- Potential specific miRNAs are suggested as possible markers and therapeutic targets for muscle-related diseases, which can be considered for future studies.
- Although the actual details of how specific miRNAs affect muscle-wasting mechanisms related to different pathophysiological conditions are limited, the current knowledge concerning which miRNAs are involved in each of the major disease area is summarized.
- In the future, as the literature in this field expands, more targeted, systematic reviews would be useful to explore detailed facets of the muscle miRNA literature, such as specific markers of the onset of muscle wasting following immobilization.
Introduction

Skeletal muscle is critical for health throughout life. Loss of muscle mass is typically seen with ageing and occurs in many chronic diseases including cancer cachexia, osteoarthritis (OA), and type II diabetes mellitus. It can also present itself more acutely through disuse atrophy, evident as a result of bed rest and limb immobilization. All muscle atrophy conditions share common functional consequences, through the loss of muscle strength and power, reducing the ability to perform activities of daily living, with a resultant impact on a person’s quality of life. These are major causes of morbidity and are poor prognostic factors for many patients. The pathogenesis of muscle wasting appears to be multifactorial, involving inflammation, impaired muscle regeneration, oxidative stress, senescence, and apoptosis. Studies suggest that these complex cellular processes play a role in myofibre degeneration followed by satellite cell activation and differentiation. The muscle regenerative response consists of activation of quiescent satellite cells, proliferation of the myogenic precursor cells (myoblasts), and terminal differentiation into myocytes and their fusion into myofibres. However, failed myofibre regeneration and/or accelerated pathological processes that affect cellular homeostasis eventually lead to muscle atrophy. Therefore, an understanding of the formation of muscle (myogenesis), but also an understanding of molecular mechanisms underpinning muscle wasting, is critical to the development of prognostic or therapeutic biomarkers.

MicroRNAs (miRNAs) belong to a class of small non-coding RNA species that post-transcriptionally alter gene expression by increasing translational repression or by inducing messenger RNA (mRNA) cleavage and degradation. They are involved in a variety of biological processes through their regulatory function in mechanisms of post-transcriptional gene expression (Table 1). Cell and animal studies have demonstrated that miRNAs are altered during muscle cell proliferation, differentiation, apoptosis, and regeneration. In recent years, miRNAs have emerged as regulators of skeletal muscle function and aberrant expression of miRNAs is thought to be associated with the progression of muscle atrophy in various diseases. For example, miR-29b has been reported to be commonly upregulated in multiple types of muscle atrophy. miRNAs have been shown to play an important role in human embryonic myogenesis and also in adult myogenesis after injury. Individual miRNAs have been shown to be involved in myogenesis, through the control of satellite cell quiescence (miR-195, miR-497), proliferation (miR-133, miR-27), and additionally myoblast differentiation (miR-206, miR-1, miR-486). In addition, miRNAs are secreted into extracellular fluids. Circulating miRNAs are attractive potential biomarkers for a variety of muscle atrophy conditions, which can also serve as signalling molecules to mediate intracellular communications. As such, these may be attractive prognostic and therapeutic biomarkers, which could serve as adjunct tools in the management of patients with muscle atrophies. This review describes the biogenesis and function of miRNAs, and highlights their role in skeletal muscle differentiation and regulation of muscle atrophy. The format of our review offers a perspective on the role of miRNAs in diseases related to muscle dysfunction investigated in different models. Due to a relatively low number of available publications, our study has used a narrative review format, which has allowed us...
MicroRNA (miRNA) biogenesis pathway. miRNAs are transcribed in the nucleus, generating primary miRNAs (pri-miRNAs) that undergo nuclear cleavage to form precursor miRNAs (pre-miRNAs). In the cytoplasm, pre-miRNAs are further processed by the Dicer. The double-stranded RNA duplex unwinds and then the mature single-stranded miRNAs assemble into RNA-induced silencing complex (RISC). The miRNA targets messenger RNA (mRNA) to either inhibit mRNA translation or induce mRNA cleavage and degradation.

MicroRNA biogenesis and function. MicroRNAs are transcribed in the nucleus by the RNA polymerase II enzyme to produce a primary-miRNA transcript (pri-miRNAs), which can be several hundred base pairs in length. Pri-miRNAs form specific hairpin secondary structures and enter a microprocessor complex, which contains ribonuclease III enzyme Drosha, which cleaves pri-miRNAs at the 3' and 5' end, leaving an approximately 70-nucleotide stem-loop precursor miRNA (pre-miRNA). Pre-miRNAs are exported from the nucleus to the cytoplasm via EXPORTIN 5, a nuclear transport protein, which recognizes double-stranded RNAs. In the cytoplasm, pre-miRNAs are further processed by the endoribonuclease Dicer to a short double-strand miRNA duplex, leaving a short double-stranded RNA duplex, which harbours the mature miRNA. The double-stranded RNA duplex unwinds and then the mature single-stranded miRNA associates with the RNA-induced silencing complex (RISC). This RISC consists of multiple proteins and when it binds with the miRNA it forms the miRNA-induced silencing complex (miRISC). As part of the miRISC, miRNAs can then interact with the untranslated region 3' (3'UTR) of target messenger RNAs (mRNAs) and either inhibit their translation or induce mRNA cleavage and degradation (Figure 1).

Muscle stem cells. Skeletal muscle has a high regenerative capacity in response to injury and exercise. This regenerative potential is fulfilled by myogenic precursors known as satellite stem cells (SSCs), which express characteristic paired-box transcription factors, such as Pax7 and Pax3. SSCs reside beneath the basal lamina, closely juxtaposed to the plasma membrane, and are mitotically quiescent in adults. They are activated in response to stimulation (such as muscle injury or exercise) by re-entering the cell cycle. This results in satellite cell proliferation and myofibre regeneration. It has been demonstrated that during this time SSCs encounter different fates (Figure 2). The majority are a fast-dividing population of Pax7+MyoD+ cells that undergo limited replication before myogenesis and are committed to differentiation, whereas a minor population of...
Fig. 2
Satellite stem cell differentiation process. In healthy adult muscle, satellite cells remain in a nonproliferative, quiescent state. They are activated in response to muscle injury or exercise. Activated satellite cells proliferate, undergo self-renewal, and differentiate into myoblasts and then to myocytes, which can mutually fuse and generate myotubes. During this time, the satellite stem cells encounter different fates. Quiescent satellite cells are characterized by the expression of transcription factors Pax7 and Pax3, whereas activated satellite cells coexpress Pax7 and myogenic differentiation factors Myf5 and MyoD. Activation of MyoG and Mrf4 characterizes the terminally differentiated myocyte. Diseases can impair satellite cell activation and proliferation, resulting in the inhibition of terminal differentiation.

slow-dividing Pax7−MyoD− cells revert to quiescent self-renewing cells.12,13

Role of miRNA in controlling skeletal muscle differentiation
Myogenesis is an ordered multi-step process during which activated SSCs stop proliferating, undergo differentiation, and fuse into myotubes (Figure 2).20 This involves a complex of transcriptional mediators but also non-coding RNA molecules. Satellite cells maintain the myogenic regulatory factors (MRFs), which are required for myogenic determination. Primary MRFs, such as myogenic differentiation (MyoD) and myogenic factor 5 (Myf5), act as determination genes, which initiate an early stage of myogenesis and characterize proliferating progenitor cells, known as myoblasts. Secondary MRFs, myogenin (MyoG) and myogenic factor 6 (Mrf4), are specific to late-stage myogenesis and characterize the terminally differentiated myocyte. In addition, the MRF family interacts synergistically with myocyte enhancer factor 2 (Mef2) to activate skeletal muscle-specific transcription promoters. Expression and splicing changes of Mef2 proteins isoforms Mef2A, Mef2C, and Mef2D in response to MyoD are consistent with the observation that the majority of skeletal muscle genes require both MyoD and Mef2 family members to activate myogenesis. Therefore, Mef2 appears to play a specific role in early events of cell differentiation. The whole process is followed by fusion into regenerating fibres. Disruption of this network entirely abrogates skeletal muscle formation, regenerative potential, and remodelling.

Recent studies have incorporated miRNAs into the complex myogenic regulatory network. Within skeletal muscle, the 'muscle-specific' miRNAs (e.g. miR-1, miR-133a, and miR-206) play a central role in muscle biology and are all induced during differentiation of myoblasts into myotubes. However, regulation between myogenic transcription factors and miRNAs is complex and depends on the cell cycle and fusion stages. For instance, overexpression of miR-1 or miR-206 in myoblasts leads to accelerated differentiation into myotubes accompanied by a decrease in cell proliferation.17,19 The overexpression of miR-1 leads to decreased levels of histone deacetylase 4 (HDAC4) protein in C2C12 cells,17 and the overexpression of HDAC4 has been demonstrated to repress both myoblast differentiation and Mef2 dependent transcription.21 Conversely, overexpression of miR-133 promotes myoblast proliferation and decreases serum response factor (SRF) levels, thus preventing SRF-mediated inhibition of myoblast proliferation.17 MicroRNAs also play a modulatory role in the upstream regulation of muscle stem cell factors such as Pax3 and Pax7; miR-1, miR-27, and miR-206 all inhibit Pax3,18,19 whereas miR–1 and miR-206 repress Pax722,34 and thus promote the terminal differentiation of myoblasts downstream of MyoD. However, the inhibition of miR-27b maintains Pax3 expression and delays differentiation. While some miRNAs are upregulated during the first stage of differentiation, others, such as miR-221 and miR-222, are downregulated,24 which is correlated with elevated expression of the cell cycle inhibitor p27 (the target of both miR-221 and miR-222). Overexpression of miR-221 and miR-222 delays cell cycle withdrawal and differentiation.24

More recently, an abundance of miRNA-489 has been identified in quiescent satellite cells and it has been shown that this is quickly downregulated during satellite cell activation, while satellite cells that lack a functional miRNA pathway spontaneously exit the quiescent stage and enter the cell cycle.23 Furthermore,
miR-195 and miR-497 have been shown to play a role in the maintenance of quiescence in juvenile muscle stem cells, by targeting the cell cycle genes, cell division cycle 25 (Cdc25), and cyclin d2 (Ccn2).15 These findings are particularly relevant to the development of muscle stem cell therapies, as manipulation of these miRNAs prior to transplantation may prompt more efficient regeneration of muscle. A novel miR-146b has also been found among the miRNAs that are upregulated during satellite cell activation and myoblast differentiation. It was proposed that its upregulation is accompanied by downregulation of the target genes such as Smad family members 4 (Smad4), notch receptor 1 (Notch1), and high mobility group at-hook 2 (Hmg2a) in order to allow the activation of myogenic differentiation programme.23 It is believed that miR-26a and miR-146b act together to regulate Smad4 during myogenesis. In vitro cell culture and animal studies established that miR-26a promotes muscle differentiation and regeneration by downregulating, signalling pathways that inhibit differentiation, (TGF-/BMP) as well as smad family member 1 and 4 (Smad1, Smad4), by targeting specifically 3’ UTRs.35

A recent study has not only established the role of miR-26a for skeletal muscle development in vivo but also its regulatory role in muscle differentiation and regeneration through myogenesis repression.15 Furthermore, recent in vitro and in vivo (rodent) studies have shown that miR-27a/b plays a role in: 1) the activation of satellite cells and their self-renewal; 2) myoblast proliferation; and 3) accelerated muscle regeneration through negative regulation of myostatin (Mstn).16,17 The loss of Mstn activates satellite cells, increasing muscle growth and regeneration, whereas its increased level leads to muscle atrophy through protein degradation and blocked protein synthesis.16 Associated with the loss of Mstn, there is myoblast activation with an increased number of Pax7 and MyoD positive cells, followed by miR-27a activation due to loss of Mstn. On the other hand, it has also been shown that miR-27 downregulates Pax3 protein levels without affecting the level of Pax7.18 However, Mstn has also been shown to be a negative regulator of Pax7 expression, therefore the increased number of Pax7 positive cells are more likely due to miR-27-mediated inhibition of Mstn rather than to direct regulation of Pax7 by miR-27. Furthermore, in vivo skeletal muscle treatment with miR-27a/b-specific antago-miRs has been shown to result in significant reduction in the numbers of Pax7 cells and activated myoblasts (MyoD), which further confirms that miR-27 is capable of Mstn regulation.16 Satellite stem cell impairment is consistent with Smad3 loss associated with increased levels of Mstn and decreased levels of miR-27, reported in Smad3-null mice. It has been shown that treatment with Mstn increased miR-27a/b expression, which was dependent on the activity of Smad3. Taken together, this evidence indicates that there is an autoregulatory mechanism in which Mstn, via Smad3 signalling, regulates miR-27a/b expression, which is responsible for satellite activation during muscle regeneration.16 To date several miRNAs have been identified that play a role in muscle stem cell activation and myogenesis, which offer the future prospect of being able to activate myogenesis directly. However, it will be important for further work to provide evidence that manipulation of these miRNAs can reduce or reverse muscle injury and muscle wasting in pre-clinical models.

**Role of miRNA in skeletal muscle in ‘in vitro’ models**

**Inflammatory cytokine-induced muscle atrophy.** The atrophic loss of muscle mass can be triggered by tumour necrosis factor (TNF-α) and insulin-like growth factor (IGF) that regulate myogenesis through complex signalling pathways that affect muscle gene expression. The physiological role of TNF-α links nuclear factor kappa B (NF-κB) with its inhibitory effect on muscle differentiation through the negative regulation of myogenic regulatory factors (MRFs). Conversely, IGF activates NF-κB, which increases both myoblast proliferation and differentiation. A recent in vitro study showed that the TNF-α inhibitory effect on muscle myogenesis is mediated by regulatory miRNAs.16 A small number of miRNAs are regulated by TNF-α or IGF1. TNF-α stimulates expression of miR-155, which is repressed during myoblast differentiation, but overexpression of miR-155 inhibits myotube formation. TNF-α induces miR-503 downregulation during myoblast differentiation whereas miR-503 expression is upregulated during myotube formation. Parallel inhibition of miR-155 and overexpression of miR-503 protects against the inhibitory effect of TNF-α on myotube formation. It is postulated that intervention at the miRNA level may reduce the inhibitory effect of proinflammatory TNF-α and thereby indirectly induce myoblast differentiation. TNF-α-induced suppression of myoblast differentiation was demonstrated to be relieved by overexpression of miR-1, miR-206, and miR-133a/b in the murine C2C12 cell-line.37 This beneficial effect can be considered as an innovative therapeutic strategy in the context of skeletal muscle atrophy. It has been suggested that biogenesis of some miRNAs is modulated by both TNF-α and IGF1 via mitogen-activated protein k (MAPK) signalling pathway and that this might have therapeutic significance.16 TNF-like weak inducer of apoptosis (TWEAK) is an inflammatory cytokine, which belongs to the TNF super lidang family, but is more potent than its structural counterpart TNF-α. TWEAK treatment of C2C12 myotubes resulted in the inhibition of miR-1 to 1, miR-1 to 2, miR-133a, miR-133b, and miR-206, while miR-146a and miR-455 were increased.38 Collectively the gene targets of miRNAs modulated by TWEAK are involved in the inflammatory response, fibrosis, extracellular matrix remodelling, and proteolytic degradation.18
The involvement of microRNAs (miRNAs) in muscle atrophy associated with cancer cachexia, sarcopenia, prolonged inactivity, and dexamethasone-induced atrophy. MicroRNA-23 and miR-206 downregulation affects the expression of transcription factor FoxO7 in satellite stem cells, promoting muscle mass wasting during prolonged activity. Following dexamethasone treatment, miR-182 suppresses forkhead box O3 (FOXO3) at the messenger RNA (mRNA) and protein level, which induces atrophy. In sarcopenia, miR-29, miR-125b, miR-143-3p, and let-7 have been shown to impair myogenesis by targeting insulin-like growth factor 1 (IGF-1) and cyclin-dependent kinase 6 (CDK6). In mice muscle, miR-431 was overexpressed and the muscle has shown reduced Smad4 level, which increases during ageing. Interactions between miRNA and mRNA have been identified in cancer cachexia muscle wasting. They include miR-199a/caveolin 1 (Cav1), miR-199a/transcription factor jun-B (junb), miR-27a/FOXO1, miR-145a/FOXO1, miR-27a/myocyte enhancer factor 2 (Mef2C), and miR-27b/Mef2C. STAT3, signal transducer and activator of transcription 3, is a corticosteroid used for the treatment of a various inflammatory-related conditions, as well as being an adjuvant treatment in cancer patients undergoing chemotherapy. Dexamethasone is widely used in vitro muscle systems to induce muscle atrophy. A number of studies have reported that miRNAs play a role in dexamethasone-induced atrophy in cultured muscle cells. High-throughput analysis of miRNAs using microarrays indicates there are multiple candidate miRNAs which are altered by dexamethasone-induced atrophy. Dexamethasone treatment of C2C12 myotubes led to the upregulation of 11 miRNAs and downregulation of six miRNAs. Independent validation by stem-loop real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) confirmed six miRNAs were differentially expressed including miR-1, miR-147, miR-332, miR-351, miR-503, and miR-708. The targets of these miRNAs include many muscle growth and atrophy-related genes. Another study showed that transfection of miR-182 into C2C12 myotubes treated with dexamethasone significantly suppressed forkhead box O3 (FOXO3) transcriptional factors at the mRNA and protein level (Figure 3). Furthermore, transfection of miR-182 which targets the 3’ UTR of FoxO3, also resulted in the down-regulation of multiple targets which are transcriptionally regulated by FoxO3, including F-box protein coding genes (Mafbx and Atrogin-1) among others. Intriguingly, extra-cellular miR-182 levels were increased in the cell culture media following dexamethasone treatment of C2C12 myotubes, which suggests miR-182 may be actively or passively released during glucocorticoid-induced atrophy.

**Role of miRNA in immobilization**

**Localized immobilization-induced muscle atrophy.** Regular mechanical loading is essential for the maintenance of muscle mass and function. Limb immobilization or hind limb suspension leads to localized skeletal muscle atrophy. This model represents changes within muscles, which may appear after the conservative treatment of long bone fractures using plaster casts. High-throughput profiling of miRNAs in the soleus muscle after two to seven days hind limb suspension in an animal model revealed changes in 18 miRNAs. Reduced miR-499 and miR-208b were identified as potential regulators of the muscle atrophy. Both of these markers were reduced even further after longer periods (observed up to 28 days) of hind limb suspension. Conversely, expression of miR-499, which targets SRY-box transcription factor 6 (Sox6), was increased. Sox6 target is established as negative regulators of β-myosin heavy chain (B-MHC), which encodes the contractile protein of the myosin heavy chain. Another study identified that the miR-30 family was downregulated by hind limb suspension. Similar changes in the miR-30 family were observed in dystrophic Mdx4cv compared to wild-type mice. SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 2 (Smardc2), snail family transcriptional repressor 2 (Snai2), and trinucleotide repeat containing adaptor 6a (Trnc6a) were validated as miR-30 family targets, which may indirectly modulate other myogenic miRNAs such as miR-206. Muscle unloading also occurs under zero gravity conditions: one previous study revealed that 11 days of spaceflight resulted in transcriptional modulation of several hundred genes, notably miR-206 expression in gastrocnemius was found to be significantly decreased.

**Bed rest-induced muscle atrophy.** Bed rest and lack of all physical activity in comparison with limb immobilization can induce well-recognized systemic metabolic changes such as decreased cardiovascular function, decreased muscle function, and increased insulin resistance. This model can be used to mimic physiological changes and the consequences of sustained inactivity and unloading, which occurs in some patients after trauma. Analysis of the vastus lateralis muscle in six males after ten days of bed rest showed downregulation of miR-206, a factor responsible for muscle function maintenance, and also a downregulation of miR-23a, a factor responsible for insulin messaging and defense against muscle atrophy (Figure 3). However, it has been shown that resistance training induces miR-206 upregulation.
and thus activates SSC proliferation, enhancing muscle differentiation.\textsuperscript{50} Downregulation of miR-23 shows an overlap with the dexamethasone-treated patients where the expression of this miRNA was also decreased, highlighting the role of this miRNA in muscle homeostasis.\textsuperscript{46} Rezen et al\textsuperscript{62} has shown that several members of the let-7 family, which affects the cell cycle and insulin resistance, were also downregulated. However, another study on vastus lateralis muscle samples, after a 21-day bed rest period, showed that the let-7 family of miRNAs was upregulated. This miRNA group is increased in type II diabetes mellitus and is associated with inactivity.\textsuperscript{51} Furthermore, the let-7 group is upregulated in muscle samples from the elderly, suggesting an overlap of the mechanisms involved in the response to unloading and ageing.\textsuperscript{52} Patients in intensive care units (ICUs) are characterized by long-term immobilization, bed rest, and artificial ventilation, leading to accelerated muscle wasting. Respiratory muscle is particularly susceptible to wasting, when artificial ventilation is necessary. One clinical study has assessed miRNA expression in muscle of septic ICU patients, which reported an increase in primary miR-21 levels, but not in its mature form of miR-21, which suggests that impairment of miR-21 processing may occur in muscles of ICU patients.\textsuperscript{53} This group of patients represents both bed rest-induced as well as cytokine-induced muscle atrophy.

**Role of miRNA in skeletal muscle atrophy**

**Cancer cachexia.** Cancer cachexia is a complex syndrome that affects patients with advanced cancer and impacts adversely on quality of life, morbidity, and mortality.\textsuperscript{44} In cancer patients, there is a wide variation in muscle atrophy and some patients appear to be more susceptible to cachexia. New interactions between miRNA and mRNA have been identified in cancer cachexia muscle wasting. These include miR-27a\textemdash FOXO1, miR-27a\textemdash Me2C, miR-27b\textemdash Cxcl12, miR-27b\textemdash Me2C, miR-140\textemdash Cxcl12, miR-199a\textemdash caveolin 1 (Cav1), and miR-199a\textemdash junb.\textsuperscript{45} MicroRNA-27a and miR-27b have been shown to regulate Me2, the gene responsible for activation of skeletal muscle-specific transcription promoters during muscle regeneration.\textsuperscript{45,55} MicroRNA-27b has also been shown to downregulate myostatin (Mstn), a member of the transforming growth factor β (TGFβ) family, responsible for SSC activation and myoblast proliferation. Jiang et al\textsuperscript{46} have demonstrated that an increase in miR-144a decreased forkhead box O1 (FOXO1) expression, suggesting that miR-145a should be considered as a potential regulator during muscle decline in cancer cachexia. In addition, miR-199 has been linked to Cav1 and transcription factor Jun-B (junb) regulation. Junb regulates gene expression on multiple levels, however it has been shown to interact with signal transducer and activator of transcription 3 (STAT3), which contributes to cancer cachexia (Figure 3).\textsuperscript{57} High-throughput sequencing of small RNAs in skeletal muscle from cachexia patients may further help to reveal novel therapeutic targets within muscle. However, muscle itself may also be a source of extracellular miRNAs. Microvesicles provide intracellular transport for different proteins and miRNAs, which are present in many different tissues. A study on miRNAs transported in microvesicles revealed the presence of miR-21, which was secreted by tumour cells, and which induced concomitant myoblast cell death.\textsuperscript{58} Microvesicles harbouring miR-21 were proposed to fuse with muscle cells and to activate toll like receptor (TLR7/8), leading to apoptosis.\textsuperscript{58} In this respect, repeated measurements of circulating miRNAs over several timepoints in cancer patients would be valuable for the discovery of novel miRNA biomarkers which can track muscle wasting.

**Sarcopenia.** In adult humans, muscle mass is lost at a rate of ~1%/year after the age of 30 years.\textsuperscript{59} This process is thought to decrease regenerative capacity in the muscle.\textsuperscript{2} Sarcopenia is defined as an age-related muscle decline. It develops by itself and often progresses independently of coexisting comorbidities that are associated with the chronic inflammatory state.\textsuperscript{60} The role of miRNA in ageing muscle is complex and confounded by factors such as physical activity. A total of 26 miRNAs have been identified to be regulated by age and exercise, but the interaction of these factors has also been shown to affect miRNA expression.\textsuperscript{61} Investigation of the role of miRNAs in sarcopenic patients has shown up regulation of both has-miR-34a-5p and has-miR-449b-5p. These miRNAs significantly increased the expression of a key senescence gene sirtuin 1 (SIRT1) and other genes related to the mitogen-activated protein kinase (MAPK) pathway, which regulate ageing processes.\textsuperscript{62} Additionally, IGF-1 and cell cycle regulators such as cyclin-dependent kinase 6 (CDK6) are downregulated in ageing muscles. MicroRNA-29, miR-125b, miR-143 to 3 p, and let-7 have been shown to impair myogenesis by targeting IGF-1 and CDK6 (Figure 3).\textsuperscript{43}

Similarly, transforming growth factor β (TGF-β) gene has been shown to play an important part in sarcopenia and to be excessively activated in skeletal muscle ageing.\textsuperscript{44} It has been demonstrated that miR-26a, miR-675, miR-146b, and miR-431 inhibit TGF-β signalling and improve muscle regeneration. Interestingly, when miR-431 was overexpressed in mice, it improved myogenic capacity of myoblasts. Likewise, when it was injected into mice muscle, the muscle has shown reduced Smad family member 4 (Smad4) levels, which increase during ageing (Figure 3). In conclusion, this study highlighted a possible role of miR-431 as a therapeutic target in muscle ageing.\textsuperscript{44}

**Role of miRNA in related disorders**

**Osteoporosis.** Osteoporosis is the most common metabolic bone disease, characterized by decreasing bone quantity over time, leading to decreased bone strength. Like sarcopenia, osteoporosis is an age-related disease.
and there is a growing consensus that the two diseases share common pathways including reduced anabolic hormone sensitivity, increased cytokine activity, and possibly even miRNA expression patterns. The role of miRNA in bone homeostasis and their effect on osteoblast or osteoclast differentiation, function, and apoptosis has been established. Some miRNAs (including miR-21 and miR-148) have been suggested as biomarkers for early diagnosis of osteoporosis. However, no specific diagnostic or predictive miRNA has proven to be efficient in clinical practice. As discussed, miR-21 has been shown to be upregulated in muscles of immobilized ICU patients and it has been shown to be a miRNA of interest in cancer cachexia. These results suggest that miR-21 plays an important role in homeostasis of the musculoskeletal system, however more research is required to understand its specific function in distinct muscle atrophies.

Osteoarthritis. Osteoarthritis (OA) is a multifactorial disease characterized by joint degeneration and pathological changes in musculoskeletal tissues such as the synovium, cartilage, and ligaments. Chondrocytes in OA are dysregulated, producing matrix-degrading enzymes. In short, inflammatory and biomechanical stress signalling pathways are similar and they induce and overexpress cytokines and chemokine genes. It remains controversial whether inflammatory mediators are primary or secondary regulators of cartilage damage in OA, however biomechanical stress, inflammatory changes, and muscle changes may all be linked and influenced by miRNAs. Periarticular loss of muscle bulk and function is well reported in OA, however the pathway and cross-communication between the OA joints and their effect on muscle is not well understood. MicroRNA-143 is differentially expressed in the cartilage of the elderly suffering with OA. Likewise, miR-143 was demonstrated to regulate senescence of satellite cells and adult muscle stem cells via insulin-like growth factor binding protein 5 (IGFBP5), suggesting a link between OA and muscle. Similarly, miR-181 was downregulated in sarcopenic muscle samples and a further study has demonstrated that the inhibition of this miRNA attenuates OA in animal models. Another miRNA, which suggests a link between muscle metabolism and OA, is the downregulation of miR-378 in elderly patients. This specific miRNA is responsible for muscle metabolism, myogenesis, and autophagy. Synovial samples taken from late-stage OA joints have identified changes in miR-378 expression. Interestingly, miR-378 has been proven to be a pro-bone regeneration molecule. These results cumulatively suggest that miR-378 can be considered as a factor involved in musculoskeletal homeostasis and a link between OA and the associated muscle changes. Importantly, miRNA analysis has shown that the function of miRNA is context-dependent and therefore a question arises whether miRNA-associated mechanisms of muscle wasting are similar or different in OA. To date many individual miRNAs have been identified to influence the local pathogenesis within the synovium and cartilage, affecting the expression of signalling molecules including tumour necrosis factor (TNF-α), interleukin 1 (IL-1), and interleukin 6 (IL-6), however apart from the discussed associations, no clear miRNA has been identified as a systemic biomarker predicting muscular phenotype or functional status of patients.

In conclusion, there is accumulating evidence, from both in vitro and preclinical in vivo studies, that miRNAs play a key role in muscle differentiation and are important regulators of muscle atrophy. This narrative review highlights that there is no consensus on how miRNA dysregulation alters the specific muscle wasting related to different pathophysiologies and may serve as a guide for future studies in this area. There are some promising candidate miRNAs, miR-21 and miR-431, identified in preclinical studies, which now need to be confirmed in human muscle disorder clinical trials. However, interventional clinical trials investigating putative miRNA drugs have exhibited significant efficacy in a range of other health conditions, including hepatitis, pathological fibrosis, cancer, and kidney disease. Specifically, the therapeutic effect of miR-29 was studied in keloid and scar tissue formation, and miR-155 was investigated for treatment of T cell lymphoma, clearly suggesting a viable future for miRNA therapeutic agents in various diseases. Considering the notable role of miR-29 and miR-155 in the muscle, the two miRNA drugs highlight a therapeutic potential of these biomarkers for muscle-related atrophy and dysfunction.

Current research has not outlined a definitive role of specific miRNA in muscle health and disease. The majority of studies evaluating muscle decline are performed in animal (quadrupedal) models. Therefore, the discrepancies of published data do not take into account the differences in studied species, skeletal muscle biopsy sites, age of studied animals, and the physiological as well as locomotor kinematic differences between quadrupedal animals and bipedal species (humans). Moreover, the time course of miRNA changes, with the development and progression of muscle atrophy, has not been determined in a longitudinal study. To date most work compares miRNAs identified in atrophy to healthy muscle, which reflect the consequences of, rather than the cause, of atrophy.

Future studies should also consider the complicated nature of miRNAs and that confounding factors can alter up- or downregulation of these targets. In order to understand better how miRNAs affect expression and function of muscle genes, further work is needed to investigate the mechanisms which control the biogenesis of miRNA. RNA-binding proteins have been identified as the upstream modulators of miRNA, however more research is required to identify these proteins, their role on miRNA, and the extracellular cross talk between different organs.

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