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ABSTRACT

Progressive skeletal muscle wasting is a natural consequence of aging and is common in chronic and acute diseases. Loss of skeletal muscle mass and function (strength) often leads to frailty, decreased independence and increased risk of hospitalization. Despite progress made in our understanding of mechanisms underlying muscle wasting, there is still no treatment available, with exercise training and dietary supplementation improving, but not restoring muscle mass and/or function. There has been slow progress in developing novel therapies for muscle wasting, either during aging or disease, partially due to the complex nature of processes underlying muscle loss. The mechanisms of muscle wasting are multifactorial, with a combination of factors underlying age- and disease-related functional muscle decline. These factors include well characterized changes in muscle such as changes in protein turnover, and more recently described mechanisms such as autophagy or satellite cell senescence. Advances in transcriptomics and other high-throughput approaches have highlighted significant deregulation of skeletal muscle gene and protein levels during aging and disease. These changes are regulated at different levels, including post-transcriptional gene expression regulation by microRNAs. microRNAs, potent regulators of gene expression, modulate many processes in muscle, and microRNA-based interventions have been recently suggested as a promising new therapeutic strategy against alterations in muscle homeostasis. Here, we review recent developments in understanding the aging-associated mechanisms of muscle wasting and explore potential microRNA-based therapeutic avenues.

INTRODUCTION

As a function of the aging process, muscle loss, referred to as sarcopenia, is a significant cause of frailty and a contributor to mortality in the elderly population (20). Diagnosis of sarcopenia is currently based on a reduction in lean body mass (33). The European Working Group on Sarcopenia in Older People (EWGSOP) defines sarcopenia as the presence of both decreased muscle mass and reduced muscle function (strength and performance) due to the non-linearity of mass and strength, as well as muscle strength not relying explicitly on muscle mass (33). Declining muscle mass, estimated at up to 40% decrease in the cross-sectional area of the *quadriceps* between 20 and 80 years of age, is a significant contributor to impairments in mobility (73). The rate of muscle loss during aging has been reported at 1-2% loss per year after age 50, with women reported to lose muscle mass at a slower rate than men (102).

The reduction in muscle mass and strength in older people is underpinned by progressive pathophysiological changes, which ultimately perturb the maintenance of muscle homeostasis. Among proposed mechanisms of sarcopenia, progressive myofiber atrophy (with type II muscle fibers potentially more susceptible to atrophy than type I fibers), alterations in satellite cell biology and therefore defective regeneration, adipose tissue infiltration and chronic inflammation have been proposed (19, 20, 97, 113, 161). Degeneration of neuronal cells and alterations in neuromuscular junction (NMJ) morphology, similarly as in neuromuscular disorders such as amyotrophic lateral sclerosis (ALS), also play a critical role in mediating the loss of muscle during aging (21, 60, 80, 97, 144). At the cellular level, satellite cell senescence and defective autophagy have been suggested important players in muscle wasting (62, 137). The mechanisms underlying sarcopenia have been comprehensively discussed in a review by Larsson et al. (80). Below, we focus on selected, some only recently proposed, mechanisms of muscle wasting.

MECHANISMS OF MUSCLE WASTING

Muscle protein homeostasis.

Skeletal muscle homeostasis depends on an intricate balance between muscle hypertrophy, atrophy and regeneration. Muscle hypertrophy and atrophy are both independent, but overlapping, active processes controlled by specific signature pathways and transcriptional programs (134). During aging and in disease, the balance between anabolic and catabolic processes in muscle is perturbed, resulting in a loss of muscle mass and function (2, 20, 40, 97, 134).

The primary anabolic pathway in muscle protein synthesis is the activation of serine/threonine kinase AKT by phosphoinositide 3-kinase (PI3-k/AKT) which upregulates mammalian target of rapamycin (mTOR) and is itself regulated by factors such as insulin-like growth factor-1 (IGF-1), exercise, hormones and amino acid intake (3, 124). The mTOR signalling pathway is a primary transducer of anabolic signalling in muscle and is required for cell growth, proliferation and suppression of autophagy (126). It has been suggested that the reduced mTOR signalling in sarcopenia does not affect muscle protein synthesis, but rather contributes to insulin and anabolic resistance following stimuli such as mechanical stress, insulin, and nutrient availability (87, 126). Contrastingly, skeletal muscle catabolism is activated by ubiquitin proteasome pathway (UPP) under the control of Forkhead-O (Fox-O) and nuclear factor-kappa B (NF-κB) (3, 4, 123, 139). Interestingly, these pathways are a part of physiological responses to stimuli such as exercise, however their dysregulation can result in muscle wasting. For example, NF-κB activation is a part of adaptation of muscle to different stressors, such as mechanical stress, however its persistent activation has been associated with muscle wasting through ubiquitin-proteasome pathway or cytokine and chemokine release (139). Other key catabolic pathways in muscle include the myostatin pathway, autophagic-lysosomal proteolysis and mitochondrial dysfunction (3, 44, 62, 89). Imbalanced protein synthesis and degradation result in either muscle hypertrophy, such as during resistance exercise, or atrophy, such as in sarcopenia, respectively. While the net loss of muscle may result in similar clinical presentation, differences exist in the underlying mechanisms contributing to muscle loss.

Autophagy

A more recently described mechanism associated with sarcopenia, is macroautophagy (here referred to as autophagy). Autophagy is a highly conserved process of degradation of cytoplasmic components of the cells, such as damaged organelles or protein aggregates, often considered a cellular “recycling” machinery (89). During autophagy, cytoplasmic cargo is delivered to the lysosome through an intermediate double membrane-bound vesicle (autophagosome) that fuses with the lysosome to form autolysosome. Autophagy is critical to the function of cells and is activated upon stimuli such as amino acid deprivation or cellular stress (15). Dysfunctional autophagy in older people and rodents has been suggested to lead to sarcopenia (69). Dysregulated autophagy is thought to be responsible for the accumulation of damaged organelles and proteins (125). Mitophagy, selective autophagy leading to breakdown of mitochondria, has been suggested to lead to increase in reactive oxygen species (ROS) production (163), which in turn has been proposed to contribute to muscle wasting in both aging (sarcopenia) and disease (163). Moreover, oxidation-dependent protein alterations have been observed in people with muscle loss (163). Recent literature further supports a relationship between ROS, autophagy and muscle weakness; while the mechanisms are not completely understood, it is suggested ROS may lead to de-regulation of autophagy (163).

Senescence

Elevated ROS levels are also thought to be associated with cellular senescence (108). Senescent cells have been suggested to contribute to muscle wasting through senescence-associated secretory phenotype (SASP) and increased “signalling noise” (8). Moreover, it has been demonstrated that removal of senescent cells can restore tissue homeostasis during aging, therefore suggesting that targeted removal of senescent cells may provide new therapeutic opportunities for aging-associated muscle wasting (10). Whilst senescence is a phenomenon mainly associated with mitotic cells, the extent of senescence in skeletal muscle, a post-mitotic tissue is debatable, with some reports demonstrating elevated levels of senescence-associated proteins in skeletal muscle from old mice (63). Interestingly, it has been

shown that satellite cells can undergo senescence resulting in their decreased regenerative potential, which may also contribute to sarcopenia development (137).

Muscle regeneration

Adult skeletal muscle regeneration is largely dependent on satellite cells, the muscle stem cell population (90). The availability and functionality of satellite cells determine effective regeneration and changes in satellite cell number have been demonstrated with aging in human and rodents (13, 18, 31, 72, 152). Moreover, human and rodent satellite cells from muscle from older individuals show transcriptional profile switch, dysregulated autophagy and reduced regenerative potential (16, 68, 91, 138). More recently, it has been demonstrated that satellite cells undergo irreversible senescence during aging, thus contributing to the reduced regenerative potential of muscle in older individuals (137). It has been therefore proposed that changes in satellite cells are key to sarcopenia development (13). However, it has to be noted that satellite cells have been shown to be dispensable in muscle hypertrophy (96) and the role of satellite cells in sarcopenia has been suggested to be negligible by others (49). Therefore, the degree to which satellite cells contribute to the development of sarcopenia remains to be established.

Fibro-adipogenic progenitor cells

Fibro-adipogenic progenitors (FAPs) are a recently described population of mesenchymal progenitor cells resident in the interstitial space of the skeletal muscle fibers which are capable of differentiation into both adipocytes and fibroblasts(70, 141). FAPs are characterized by the expression of PDGFRα and other markers such as Sca1 (only in mice) and CD34. FAPs are thought to be key for successful muscle regeneration and repair in healthy and young individuals (107). During normal muscle regeneration, FAPs proliferate and release signals, such as IL-6, to stimulate satellite cell differentiation (117) (107). This regenerative potential might be also enforced by the differentiation of FAPs into fibroblasts, as fibroblasts are necessary for connective tissue repair of the extracellular matrix (ECM) (81). However, during defective muscle regeneration in aging or disease, fibrotic scar may be formed, followed by adipose tissue infiltration. This “fatty degeneration” is characteristic of sarcopenic muscles (20, 107). Fatty degeneration and fibrosis are mediated via TGF-β and PDGFRα signalling pathways and by the activation of senescence-related markers such as p21 and

184 $p16^{INK4a}$ in myofibroblasts (71, 99). Furthermore, uncontrolled activation and
185 differentiation of FAPs into fibroblasts and adipocytes during muscle wasting in aging
186 and dystrophic diseases have been demonstrated. This results in the infiltration of fatty
187 tissue and fibrotic scars, thus limiting the proper regeneration and repair of the muscle
188 (107, 142). Interestingly, it has been suggested that the presence of senescent FAPs
189 in $Bubr1^{H/H}$ mice, a premature aging model, rather than senescent satellite cells, may
190 be a driver of muscle aging (9). Therefore, age-related changes in the function of FAPs
191 may be a yet undescribed mechanism contributing to sarcopenia development.

192 **MICRORNAs IN MUSCLE WASTING**

193 microRNAs (miRNAs, miRs) are short, non-coding RNAs that regulate gene
194 expression post-transcriptionally (5). Over 2000 microRNAs have been discovered in
195 humans and it is believed that miRNAs are predicted to regulate two-thirds of the
196 human genome, suggesting that miRs modulate many physiological processes (48,
197 59). miRs have been strongly implicated in regulating muscle development and
198 homeostasis (56). Mature miRNAs are generated from primary (pri-miRNA)
199 precursors, which are cleaved in the nucleus by the enzyme Drosha to form the pre-
200 miRNA transcript (79). The pre-miRNA is transported into the cytoplasm and cleaved
201 by the enzyme Dicer to generate a 19–24 base pairs long miRNA duplex (11). This
202 duplex is unwound and the mature miRNA strand is incorporated into a protein
203 complex called RISC (RNA Induced Silencing Complex). The non-incorporated strand
204 is often degraded, however in some cases it may be incorporated into the RISC
205 through the Argonaute (AGO) proteins, a family of proteins which bind to small non-
206 coding RNAs and guide them into the RISC complex. miRNAs guide RISC to partially
207 complementary sequences, usually contained within the 3' UTR of target mRNA
208 transcripts. The activity of RISC can be modulated by mRNA-binding proteins which
209 can, depending on cellular context, prevent or activate microRNA-mediated repression
210 (79).

211 The mechanisms of microRNA-dependent gene silencing are complex and several
212 models have been proposed (140). Traditionally, microRNAs within RISC (also
213 referred to as miRISC) pair to sites within mRNAs leading to translation inhibition by
214 deadenylation of the target mRNA(s), which can be followed by decapping and also
215 RNA degradation (140). Some of AGO-interacting proteins can accelerate mRNA

degradation through concentrating miRNA targets with mRNA silencing factors (98). Interestingly, under certain conditions such as starvation, microRNAs have been reported to enhance translation of their target mRNAs (151). The mechanism of this is not well understood, however it appears to be associated with mRNAs lacking cap and poly(A) tail (151). Furthermore, subcellular localization of microRNAs is important for miRNA function and may influence their mode of action on target mRNAs. Studies have indicated P-bodies, cytoplasmic processing bodies, as primary sites of microRNA activity (46). P-bodies have also been associated with factors involved in mRNA decay and translational repression (45) miRISC has also been found to co-localise with polysomes (rough endoplasmic reticulum, ER) in animals; this could be associated with fast and more effective miRNA-regulated regulation of gene expression in response to environmental factors (Ref 41, 44). Interestingly, several microRNAs have been detected within mitochondria (mito-miRs) (41) These miRs can be produced from the mitochondrial genome or be imported from the cytoplasm through mitochondrial intermembrane proteins (155). mito-miRs have been reported to block the translation of mitochondrial mRNAs, for example miR-181c targeting mCox1, but also to stimulate translation, for example miR-1 stimulating translation of mitochondrial mRNAs (169). The mode of action of mito-miRs and their relevance to overall tissue homeostasis remains to be established. Finally, the presence of microRNAs has been demonstrated in the nucleus of mammalian cells; these miRs were found to be associated with chromatin or present in the nucleoplasm (116). It has therefore been suggested that chromatin-associated miRs can regulate translation or splicing, whereas miRs in nucleoplasm may act through post-transcriptional silencing (140). In addition to the complexity of miRNA action due to their subcellular localisation and interacting proteins, miRNA precursors can undergo editing by adenosine deaminases that catalyze the conversion of adenosine to inosine, altering the base-pairing of transcripts. These modifications can affect miRNA processing and properties of mature miRNAs (79). Furthermore, a single microRNA can target multiple mRNAs and a single mRNA can be targeted by multiple miRs. By influencing expression of multiple target genes, miRNAs provide a robust and highly responsive mechanism that enables cells to react to changes within their immediate or surrounding environment. Given their role as novel regulators of gene expression and their vast deregulation in a variety of pathophysiological conditions, miRNAs are emerging as powerful regulatory molecules and potential novel therapeutic agents (146).

microRNA role in muscle development and regeneration

Myogenesis, a complex process of muscle formation, occurs during development as well as in adulthood during muscle regeneration. The cells involved in myogenesis include, but are not restricted to, muscle stem and progenitor cells from within the myotome, satellite cells and adult skeletal muscle stem cells (105).

Muscle-specific or –enriched set of microRNAs have been described; these include, but are not restricted to, mir: -1, miR-133, miR-206, also called “myomiRs”, and miR-208, miR-486 and miR-499 (22, 94, 132, 147). The role of microRNAs in embryonic and adulthood myogenesis has been demonstrated in Dicer, a key enzyme in microRNA maturation, knock-out mice, which show delayed myogenesis or inability of satellite cells to reach terminal differentiation (29, 55). Several microRNAs have been associated with both muscle development and disease. For example, the expression of Pax3 and Pax7, key transcription factors in early myogenesis, is regulated by miR-206 in myogenesis during development and adult skeletal muscle regeneration (32, 55). miR-1 and miR-133 have been shown to control the SWI/SNF subunit composition (chromatin remodelling complex) during development and disease (54, 103, 122) and miR-208b and miR-499 have been demonstrated to regulate myofiber type composition (149). Satellite cell depletion, senescence and functional decline have been proposed to be associated with sarcopenia development (137). Cheung *et al.* have demonstrated that mice with satellite cells depleted of Dicer show reduced muscle regenerative capacity and premature muscle wasting (29). In a different study, Let-7 family of miRNA, which regulate cellular proliferation and expression of Pax-7, a key myogenesis transcription factor, was upregulated in muscle of older people and suggested to play an important role in satellite cell functional decline during aging(40). Finally, exogenous administration of miR-1, 133 and 206 by local injection has been shown to accelerate muscle regeneration after injury in rats, suggesting that microRNAs might be used as a therapeutic strategy against muscle damage (106).

The role of miRs in regulating muscle hypertrophy and atrophy

microRNAs have been shown to maintain muscle homeostasis in adulthood, including control of muscle hypertrophy and atrophy and muscle adaptation to exercise, which are disrupted during aging (35, 143). Changes in microRNAs levels in muscle, for

example miR-133 or miR-206, are associated with a myriad of age-related degenerative pathologies, including muscle atrophy and aging (20, 27, 30, 40, 58, 75). Several miRNAs have been shown to be differently expressed in the muscle of older humans and rodents (Table 1).

Some of the miRs downregulated in muscle of aged mice, such as miR-133 or miR-181, have been shown by us and others to regulate muscle hypertrophy or atrophy through regulation of anabolic pathways and SIRT-1, respectively (61, 135). Sirt-1 plays an important role in regulating autophagy. Autophagy has been demonstrated to play a key role in maintaining muscle mass, neuromuscular communication, as well as stem cell stemness (24, 52, 88).

Anabolic resistance, a process well characterized during sarcopenia and immobilization, is the inability to effectively initiate protein synthesis following the ingestion of protein or exercise (34, 114). Several miRs have been shown to be upregulated following exercise and following protein ingestion (23, 39, 86, 110). Drummond *et al* have shown decreased expression of miR-1 expression in the muscle of younger, but not older, individuals following exercise, whereas the primary but not mature miR-133a and miR-206 transcripts were differentially regulated in the muscle of young and older people (39). These data suggest that the lack of change in miR-1 expression in the muscle of older people following exercise and protein ingestion may contribute to anabolic resistance during aging.

With more data emerging on the capability of microRNAs to regulate muscle mass, it is possible that microRNA-based approaches could be developed into therapeutic approaches in the future.

miRs and neuromuscular communication

Deterioration of neuromuscular communication is an important aspect of muscle wasting in both aging and disease. The expression of miRNAs: miR-23a and miR-29b, has been shown to be increased in skeletal muscle during aging and in disease, these miRs have been associated with the disruption of the mitochondrial-related gene expression in the muscle (20, 63, 121). miR-206 has also been shown to be upregulated in a mouse model of amyotrophic lateral sclerosis (ALS) (161). ALS is the most common neurodegenerative disease in adults, characterized by muscle atrophy, denervation and paralysis. miR-206 deficiency in these mice accelerates the

progression of the disease (161). Interestingly, the authors suggest that changes in miR-206 expression are a part of compensatory mechanism aiming at improving neuromuscular degeneration rather than a part of a mechanisms driving this degeneration (36). This proposed mechanism is associated with retrograde transport from muscle to nerve. Another study investigating communication from muscle to nerve demonstrated that myofibers can release exosomes, membranous vesicles of approximately 50-150 nm in diameter with roles in cell-to-cell communication, which contain microRNAs (36). The microRNA content of these vesicles was reported to change after denervation. More importantly, microRNAs contained within exosomes released from myofibers were taken up by other cell types and this was associated with changes in miRNA and its target levels in the recipient cells (36). It remains to be established whether miR-based interventions can restore neuromuscular interactions during aging.

miRs and cellular senescence

Whilst muscle is a post-mitotic tissue, and therefore unlikely to undergo senescence, geriatric and senescent satellite cells have been demonstrated in muscle of old mice (137). Our group has demonstrated an important role of miR-143 in regulating satellite cell senescence during aging (136). Downregulation of miR-143 in satellite cells from old mice was associated with increased cell viability; however this was at the cost of cellular senescence, suggesting miR-143 downregulation in satellite cells during aging may be a part of a compensatory, rather than causative, mechanism (136).

Another miRNA, miR-29, has been characterized as a senescence-related microRNA. miR-29 is increased in the skeletal muscle during aging and enhances cellular senescence by targeting distinct pathways involved in muscle growth and satellite cell proliferation, including IGF-1, p85 and B-myb (64). However, miR-29 seems to have an anti-fibrotic activity in multiple tissues (37), and the loss of miR-29 in myoblasts contributes to the pathogenesis of Duchenne muscular dystrophy by promoting myoblast trans-differentiation into myofibroblasts (157). As the importance of senescence in skeletal muscle functional deterioration remains to be established, the potential of miRs targeting senescence-associated genes as therapeutics for sarcopenia remains elusive.

microRNA-mediated regulation of mitochondrial dynamics

The function of skeletal muscle, an energy-demanding tissue, is associated with mitochondria. Changes in mitochondrial dynamics have been previously reported and it has been suggested that dysfunctional mitophagy and mitochondrial generation may be key to muscle wasting (76). Several miRs have been shown to regulate the mitochondrial homeostasis and cellular metabolism (83) (25, 130). For example, miR-696 has been shown to target PGC-1α, a transcription factor key to mitochondrial biogenesis, and its downstream effectors; pyruvate dehydrogenase kinase-4 (PDK4) and cytochromes c oxidase subunit II (COXIV) (7). miR-696 overexpression led to decreased fatty acid oxidation (7). Another study has shown that miR-133a-deficient mice have lower levels of PGC-1α and NRF1 and decreased mitochondrial mass and exercise tolerance (109) This is phenotypically similar to sarcopenia, suggesting the role of miR-133a in maintaining mitochondrial dynamics in skeletal muscle. Furthermore, Russell *et al.* demonstrated disrupted mitochondrial homeostasis with a decrease in the levels and activity of PGC1-α, NRF-1, COXIV, ERRα in the mouse model of ALS. Another miR, miR-23, has been shown to be upregulated in mouse models of ALS (121, 161) and the overexpression of miR-23 in wild type mice has been associated with mitochondrial dysfunction similar to the animal models of ALS, suggesting a causative effect of miR-23 elevated levels in muscle wasting during ALS (121). However, a different study found miR-23a to decrease the expression of Atrogin-1 and Murf1, ubiquitin ligases upregulated in models of muscle atrophy, with miR-23 overexpression protecting against glucocorticoid-induced atrophy (153). In addition, miR-23 expression is decreased in other models of atrophy in -rat and C2C12 myotubes (66). This suggests that the mechanisms responsible for the different models of atrophy may differ which is in line with data by Soares et al. demonstrating that microRNA function is context dependent in different models of muscle atrophy (134).

microRNAs and FAPs

Interestingly, microRNAs have also been shown to regulate the functionality of FAPs. For example, fibroblasts growth factor-2 (FGF2) has been shown to induce miR-29a expression in FAPs and in myogenic progenitors, which in turn stimulated myoblasts proliferation (50). It has also been reported that activation of the expression of

myomiRs can block adipogenesis of FAPs and enhance muscle regeneration (122). Moreover, miR-23a overexpression has been shown to reduce lipid accumulation within the skeletal muscle by inhibiting the differentiation of PDGFRα^{+} progenitor cells into adipocytes (57, 67). MyomiRs have also been shown to regulate FAP functional phenotype in dystrophic mice through regulation of BAF subunits, part of the SWI/SNF chromatin remodelling complex (122). Interestingly, regulation of BAF subunits has also been demonstrated during embryonic myogenesis (54). These data suggest that microRNAs may serve potent therapeutic tools against fibrosis and fatty degeneration during sarcopenia and disease by regulating FAPs.

microRNAs are clearly one of the important mechanisms underlying muscle wasting, and investigation of miRNA-associated mechanisms of muscle wasting will be important to understanding their full role in the progression of sarcopenia and potential in the design of novel therapeutics.

MICRORNAs AND NEW THERAPEUTIC AVENUES

Current interventions for sarcopenia and other muscle wasting disorders, such as cachexia, focus on progressive resistance training, orexigenic drugs and anabolic agents (53). Recently, a dual activin-type II receptor (ActRIIA/ActRIIB) antibody inhibitor to myostatin has been shown to enhance muscle hypertrophy in mice (104), although challenges remain with respect to human clinical trials (101). Other interventions focus on either anabolic pathways, as in androgen hormone replacement and growth hormone secretagogues such as ghrelin (53), or through inhibitory pathways such as in myostatin blocking therapies, angiotensin receptor blockers and beta adreno-receptor blocking (53, 112). While these options are being evaluated as potential treatments for ameliorating muscle loss, few clinically validated options exist, demanding a need for further investigation. Moreover, the development of novel therapies for muscle wasting is somewhat slowed down by the lack of robust and reliable non-invasive biomarkers of muscle wasting, with creatine kinase being the most commonly used assay (74). Due to their stability in biofluids and reported changes in circulating miRNA levels in various muscle disorders, miRs have been proposed novel biomarker of muscle wasting (reviewed in (74, 131)). Furthermore, as

409 miRNAs are small molecules with limited immune concerns, they are excellent
410 therapeutic candidates.

411 Investigation into miRNA therapies to treat challenging diseases remains an area of
412 ongoing interest. Two approaches are currently being used to modulate microRNA
413 activity: synthetic double-stranded miRs or viral-based miR delivery to overexpress
414 microRNAs and chemically modified antagomir oligonucleotides to inhibit microRNA
415 function (146). Several companies have developed or are working towards miRNA
416 pharmaceuticals in different fields of medical research, including cancers, metabolic
417 diseases, neurological diseases, cardiovascular disease, inflammatory diseases and
418 others (38), but few miRNA therapies have gained clinical traction (39). Indeed, over
419 1.5 thousand microRNA-associated patents for therapeutic purposes have been filed
420 and one miRNA-based therapeutic, the compound SPC3649 (Miravirsen), an inhibitor
421 of miR-122 against hepatitis C virus (Santaris Pharma, Denmark), has entered a
422 phase II of clinical trial (1, 26). Miravirsen was reported to be well tolerated with no
423 dose-limiting toxic effects or treatment discontinuations due to adverse effects (145)
424 The miR-34 mimic-based drug (MRX34) for treatment of liver cancer is currently in
425 Clinical Phase I and other miRNA-based therapeutics are in the preclinical stage (17,
426 146). These studies provide encouraging evidence that pharmacological modulation
427 of miRNA activity is feasible in human patients. However, issues remain with the use
428 of miRNA-based therapies.
429 Identifying miRNA targets of interest remains an area of critical investigation, and the
430 heterogeneity of miRNA expression continues to remain a challenge to the
431 progression of miRNA pharmaceuticals (118). Ensuring the specificity of targets will
432 also continue to be a challenge in limiting the potential of unwanted off-target effects
433 and toxicity (148). New methods for improved microRNA target validation and
434 characterisation of off-target effects are needed to progress the development of miR-
435 based therapeutics. Suitable delivery systems, including tissue-specific delivery, that
436 allow for stability and safety are also concerns facing current research in miRNA
437 therapeutics. Other issues remain to be resolved, such as understanding the long-
438 term effects of modulating microRNA activity *in vivo*, establishing efficacy and safety
439 of miR-based therapeutics in human patients, and modelling of pharmacokinetics and
440 pharmacodynamics of these molecules.

441 microRNAs are deregulated in muscle in aging and multiple disorders associated with
442 muscle wasting indicating they could be viable therapeutic targets for muscle loss.
443 Several issues may impede this development. It appears that changes in microRNA
444 expression in muscle, as well as microRNA function, can vary depending on the
445 disease underlying muscle wasting. Moreover, few studies have investigated
446 microRNA targets and potential off-targets in muscle and other tissues following
447 systemic microRNA mimic/antagomir delivery. Indeed, functional studies of
448 microRNAs in skeletal muscle during aging or disease are still in their infancy as
449 compared to miR research in diseases such as cancer. With local delivery not feasible
450 due to the size of muscle tissue, tissue-specific delivery will be very important for
451 muscle therapies to avoid delivery of miR mimics/antagomiRs into other tissues, such
452 as liver or lungs when using systemic delivery. miRNA therapies for muscle wasting
453 will also require careful optimization of sufficient but safe doses of miR
454 mimics/antagomiRs.

455 In summary, the rapidly expanding number of functional miRNA studies in muscle
456 provides a basis for development of miR-based approaches for improvement of
457 muscle mass and function. Despite large numbers of patents filed for therapeutic use
458 of miRs in different disorders, very few patents have been filed to use microRNA-
459 based approaches for treatment of muscle wasting, and none have been filed for
460 sarcopenia. Much remains to be learned about the function of miRNAs in muscle
461 wasting due to aging or disease, and the optimal delivery systems for efficient and
462 safe manipulation of microRNA activity will be key in development of miR-based
463 therapies for muscle loss. Nevertheless, the first human trials of miR-based
464 therapeutics are ongoing, providing proof-of-principle for the use of miR-based
465 therapies in human patients.
466 There will inevitably be challenges during the developments of microRNA-based
467 therapies for treatment of muscle wasting, however if these obstacles can be
468 overcome, microRNA-based therapeutics hold a potential to be the next generation of
469 drugs for muscle wasting.

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472 were obtained from www.canstockphoto.co.uk under Standard License Agreement.

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Table 1. Summary of microRNAs with known or potential role in regulation of myogenesis and muscle size and function.

microRNA	Chromosome/ Cluster (humans)	Function/Mechanism	Specie/Cell line	Reference
Let-7	Chr22: let-7a-3, miR-4763, let-7b Chr19: miR-99b, let-7e, miR-125a	Let-7b/e are upregulated during skeletal muscle aging; possibly affecting the expression of Pax7 through the repression of cell cycle regulators, impeding satellite cell self-renewal	Human	(40)
		Promotes neuronal autophagy by repressing mTORC1. Anti-let-7 resulted in increased lean and fat mass	Primary cortical neurons from GFP-LC3 transgenic mice	(42)
		Let7-b is involved in cellular senescence. MitomiR	Mouse Embryonic Fibroblasts (MEFs), precancerous cells, Skeletal muscle cells	(115)
		Let-7e is increased after denervation	Mouse	(36)
miR-1	Chr20: miR-1-1 Chr18: miR-1-2, miR-133a-1	MyomiR	Human, mouse, Human HeLa cells	(129) (12) (78)
		Downregulated in a *in vivo* model of skeletal muscle hypertrophy (7 days of functional overload of the plantaris muscle)	Mouse	(93)
		Mature miR-1 and pri-miR-1-2 are downregulated in the young men followed anabolic	Human	(39)

		stimulus, but not in the older men. At baseline, pri-miR-1-1 and pri-miR-1-2 are upregulated in the older men compared to the younger, but not the mature miR-1 (vastus lateralis muscle)		
		Sarcomeric actin organization	Zebrafish	(100)
		Increased expression 1 hour after acute exercise in untrained individuals, but downregulated at rest after chronic exercise (vastus lateralis muscle)	Human	(110)
		Local injections of double-stranded microRNA accelerate muscle regeneration. Upregulate MyoD1, myogenin and Pax7	Rat, C2C12 murine cell line	(106)
		Induces myogenic differentiation during development by targeting Pax3	Chicken embryo, rat RuGli glioblastoma Cells	(55)
		Increased expression 3 hours after acute exercise. Remains upregulated after 10 days of endurance training (vastus lateralis muscle). Aerobic exercise training restores the levels of miR-21 in the soleus muscle of spontaneously hypertensive rats	Human, rat	(119) (47)
		Downregulated by TWEAK/Fn14. TWEAK/Fn14 induces muscle mass loss	Mouse	(Sato et al. 2014) (111)
		Targets BAF60a and BAF60b, inducing myogenic differentiation	Chicken embryo, mouse	(54) (122)
		Inhibits fibro-adipogenic progenitors (FAPs) phenotype by targeting BAF60a and BAF60b	Mouse	(122)
		Involved in myoblasts differentiation	Piaractus mesopotamicus	(43)
		Higher expression in the skeletal muscle of obese rats	Rat	(82)
miR-16	Chr13: miR-15a, miR-16-1 Chr3: miR-15b, miR-16-2	Lower expression in the skeletal muscle of obese rats. Enhances autophagy and inhibits insulin-stimulated protein synthesis	Rat	(82)

		Increased after denervation.	Mouse	(36)
miR-19b	Chr13: miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, miR-92a-1 ChrX: miR-106a, miR-18b, miR-20b, miR-19b-2, miR-92a-2, miR-363	Involved in cellular senescence and inflammaging. MitomiR. Downregulated in human aging	Human cells	(115)
miR-20a	Chr13: miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, miR-92a-1	Involved in cellular senescence and inflammaging. MitomiR. Downregulated in human aging	Human cells	(115)
miR-21	Chr17: miR-21	Inhibits apoptosis, is downregulated after ischemia, is a cancer biomarker and promotes fibrosis. Positive regulator of AK signalling pathway. Aerobic exercise training restores the levels of miR-21 in the soleus muscle of spontaneously hypertensive rats	Mouse, cardiac myocytes, rat	(128) (47)
		Markedly increased after denervation	Mouse	(134)
miR-23a	Chr19: miR-23a, miR-27a, miR-24-2	Protects muscles from atrophy by targeting MAFbx/atrogin-1 and MuRF1	Mouse, C2C12 murine cell line	(154)
		Increased expression in skeletal muscle of amyotrophic lateral sclerosis (ALS) patients. miR-23a represses the expression of PGC-1α, resulting in mitochondrial dysfunction	Human, mouse	(120)
		Decreased expression 3 hours after acute exercise (vastus lateralis muscle)	Human	(119)
		Decreased during diabetes-induced muscle atrophy. Present in exosomes released from muscle cells	Rat, C2C12 murine cell line	(65)
		Increased expression 4 hours after exercise with post-exercise protein ingestion compared to placebo	Human	(23)

		ingestion (vastus lateralis muscle)		
		Inhibits the differentiation of PDGFRα+ progenitor cells into adipocytes by targeting ZNF423	Fetal bovine skeletal muscle	(57)
miR-27a-3p	Chr19: miR-23a, miR-27a, miR-24-2	Downregulated in skeletal muscle during aging	Human	(40)
		Inhibits estradiol (E_2) production and promotes apoptosis by targeting Creb1	Mouse, mouse primary ganglion cells (GCs)	(158)
		Promotes myoblasts proliferation through upregulating MyoD and myogenin and by targeting myostatin	Mouse C2C12 cell line	(28)
		Inhibits satellite cell proliferation by targeting Pax3, inducing differentiation	Goat	(84) (32)
		Increased after denervation	Mouse	(36)
		Increased expression in obesity and insulin resistance. miR-27a is released from adipocytes resulting in skeletal muscle insulin resistance by targeting PPARγ	Human, mouse, mouse C2C12 cell line	(165)
miR-29	Chr7: miR-29b-1, miR-29a Chr1: miR-29b-2, miR-29c	Promotes myogenesis and differentiation by targeting HDAC4, attenuating the negative effects of TGF-β in muscle differentiation	Mouse primary cells, mouse C2C12 cell line	(162)
		Increased expression of miR-29b in skeletal muscle of amyotrophic lateral sclerosis (ALS) patients	Human	(120)
		Inhibits proliferation and favours myoblasts differentiation by targeting Akt3	C2C12 cell line, mouse satellite cells and primary myoblasts	(160)
		Enhances cellular senescence, inhibits fibrosis and supresses tumor growth through the activation of p53 pathway. Increased expression in aged mice	Mouse, rat, C2C12 cell line, mouse primary myoblasts	(157) (64) (37)
		Increased expression of miR-29b after 10 days of endurance training (vastus lateralis muscle)	Human	(119)

		FGF2 induces the expression of miR-29a in fibro-adipogenic progenitors (FAPs) and myoblasts, promoting myogenic proliferation	Mouse, human	(50)
		miR-29b and miR-29c are increased after denervation	Mouse	(36)
miR-106a	ChrX: miR-106a, miR-18b, miR-20b, miR-19b-2, miR-92a-2, miR-363	Involved in cellular senescence. MitomiR. Downregulated in human aging	Human cells	(115)
		Increased after denervation	Mouse	(36)
miR-133a	Chr18: miR-1-2, miR-133a-1 Chr20: miR-133a-2	MyomiR	Human, mouse	(129) (12) (78)
		Downregulated in a *in vivo* model of skeletal muscle hypertrophy (7 days of functional overload of the plantaris muscle)	Mouse	(93)
		Pri-miR-133a-1 and pri-miR-133a-2 are downregulated in the young men followed anabolic stimulus, but not in the older men. At baseline, pri-miR-133a-1 and pri-miR-133a-2 are upregulated in the older men compared to the younger, but not the mature miR-133a (vastus lateralis muscle)	Human	(39)
		Sarcomeric actin organization	Zebrafish	(100)
		Increased expression after acute exercise in untrained individuals, but it is downregulated at rest after chronic exercise (vastus lateralis muscle)	Human	(110)
		Local injections of double-stranded microRNA accelerate muscle regeneration. Upregulate MyoD1, myogenin and Pax7	Rat, C2C12 murine cell line	(106)
		Downregulated in skeletal muscle during aging	Human	(40)
		Increased expression 3 hours after acute exercise (vastus lateralis muscle)	Human	(119)
		Downregulated by TWEAK/Fn14. TWEAK/Fn14 induces muscle mass loss	Mouse	(127)

		Promotes slow-to-fast muscle fibre type shifting by targeting TEAD1	Mouse, C2C12 cell line	(168)
		Inhibits fibro-adipogenic progenitors (FAPs) phenotype and promotes myogenic differentiation by targeting BAF60a and BAF60b	Mouse	(122)
		Involved in myoblasts proliferation	Piractus mesopotamicus	(43)
		Important for mitochondrial biogenesis and exercise tolerance	Mouse	(109)
		Increased after denervation	Mouse	(36)
miR-133b	Chr6: miR-206, miR-133b	MyomiR	Human, Human, mouse, HeLa cells	(129) (12) (78)
		Downregulated at rest after chronic exercise (vastus lateralis muscle)	Human	(110)
		Local injections of double-stranded microRNA accelerate muscle regeneration. Upregulate MyoD1, myogenin and Pax7	Rat, C2C12 murine cell line	(106)
		Downregulated in skeletal muscle during aging	Human	(40)
		Increased expression 3 hours after acute exercise (vastus lateralis muscle)	Human	(119)
		Muscle regeneration and development	Mouse	(14)
		Involved in cellular senescence. MitomiR	Human skeletal primary myoblasts	(115)
		Downregulated by TWEAK/Fn14. TWEAK/Fn14 induces muscle mass loss	Mouse	(127)
		Targets BAF60a and BAF60b, inducing myogenic differentiation	Chicken embryo	(54)
		Involved in myoblasts proliferation	Piractus mesopotamicus	(43)
		Increased expression 4 hours after exercise with post-exercise protein ingestion compared to placebo ingestion (vastus lateralis muscle)	Human	(23)
		Lower expression in the skeletal muscle of obese rats	Rat	(82)

		Increased after denervation	Mouse	(36)
miR-143	Chr5: miR-143, miR-145	Downregulated in satellite cells and primary myoblasts during aging. Inhibits cellular senescence by targeting Igfbp5	Human, mouse	(136)
		Increased after denervation	Mouse	(36)
miR-146a	Chr5: miR-146a	Involved in cellular senescence and inflammaging. MitomiR	Bone marrow-derived dendritic cells, dermal fibroblasts, 143B human cells	(115)
miR-181a	Chr1: miR-181a-1, miR-181b-1 Chr9: miR-181a-2, miR-181b-2	Increased after 10 days of endurance training (vastus lateralis muscle)	Human	(119)
		Involved in cellular senescence and inflammaging. MitomiR	Dermal fibroblasts, CD4 T cells, Human primary myoblasts, 143B human cells, HEK293 and HeLa	(115)
		Increased expression of miR-181 4 hours after exercise with post-exercise protein ingestion compared to placebo ingestion (vastus lateralis muscle)	Human	(23)
		Regulates myotube size by targeting Sirt-1	Mouse, C2C12 cell line	(135)
miR-206	Chr6: miR-206, miR-133b	MyomiR. Skeletal muscle specific	Human, mouse, HeLa cells	(129) (92) (78)
		Pri-miR-206 (but not the mature miR-206) is upregulated in the young and older men followed anabolic stimulus but at different time points (vastus lateralis muscle)	Human	(39)
		Involved in myoblasts differentiation	Rat	(95)
		Delays Amyotrophic lateral sclerosis (ALS) progression. miR-206 is upregulated in a mouse model of ALS although its deficiency accelerates ALS. It is needed for the regeneration of	Mouse	(161)

		neuromuscular synapses after acute injury		
		Downregulated at rest after chronic exercise (vastus lateralis muscle)	Human	(110)
		Local injections of double-stranded microRNA accelerate muscle regeneration. Upregulate MyoD1, myogenin and Pax7	Rat, C2C12 murine cell line	(106)
		Induces myogenic differentiation by targeting Pax3 and Pax7 during development and skeletal muscle regeneration in the adult	Chicken embryo, rat RuGli glioblastoma Cells, C2C12 cell line, mouse primary myoblasts	(55) (38)
		Increased expression of miR-29b in skeletal muscle of amyotrophic lateral sclerosis (ALS) patients	Human	(120)
		Downregulated by TWEAK/Fn14. TWEAK/Fn14 induces muscle mass loss	Mouse	(85)
		Enriched in slow twitch muscle fibres such as soleus	Mouse	(127)
		Fibrosis/ Duchenne	Mouse	(14)
		Promotes myogenesis and differentiation by targeting HDAC4, attenuating the negative effects of TGF-β in muscle differentiation	Piaractus mesopotamicus, mouse primary cells, mouse C2C12 cell line	(43) (162)
		Targets BAF60a and BAF60b, inducing myogenic differentiation	Chicken embryo, mouse	(54) (122)
		Inhibits fibro-adipogenic progenitors (FAPs) phenotype by targeting BAF60a and BAF60b	Mouse	(122)
		Markedly increased after denervation	Mouse, C2C12 murine cell line	(134) (36)
miR-208b	Chr14: miR-208b	MyomiR	Mouse	(150) (149)
		Enriched in slow twitch muscle fibres such as soleus. Favours slow-twitch muscle fibre conversion and increases exercise endurance. Targets Sox6. Sox6 induces a fast twitch phenotype	Rat, mouse, C2C12 cell line, mouse primary myoblasts	(95) (149) (51) (168) (167)

miR-378	Chr5: miR-378a Chr8: miR-378d-2 Chr3: miR-378b Chr10: miR-378c Chr4: miR-378d-1 Chr5: miR-378e Chr1: miR-378f Chr1: miR-378g Chr5: miR-378h Chr22: miR-378i Chr17: miR-378j	Inhibits estradiol (E_2) production by targeting aromatase	Porcine granulosa cells	(164)
		Downregulated in skeletal muscle during aging	Human	(40)
		Delays satellite cells activation through targeting Igf1r	Mouse	(166)
		Increased after denervation	Mouse	(36)
miR-486	Chr8: miR-486-2, miR-486-1	MyomiR	Mouse	(133)
		Induces hypertrophy by targeting PTEN and FoxO1	Mouse	(133)
		Induces myoblasts differentiation by targeting Pax7	C2C12 cell line, mouse primary myoblasts	(38)
miR-499	Chr20: miR-499a, miR-499b	Muscle enriched	Zebrafis, mouse	(77) (149)
		Enriched in slow twitch muscle fibres such as soleus. Favours slow-twitch muscle fibre conversion and increases exercise endurance. Targets Sox6. Sox6 induces a fast twitch phenotype	Rat, mouse, C2C12 cell line, mouse primary myoblasts, Piractus mesopotamicus, mouse primary ganglion cells (GCs)	(95) (149) (51) (168) (43) (167) (159)
miR-696	*Not described in humans	Negatively affects fatty acid oxidation and mitochondrial biogenesis by targeting PGC-1α High expression in the skeletal muscle; lower expression during myoblast differentiation	Mouse, C2C12 murine cell line	(6) (156)

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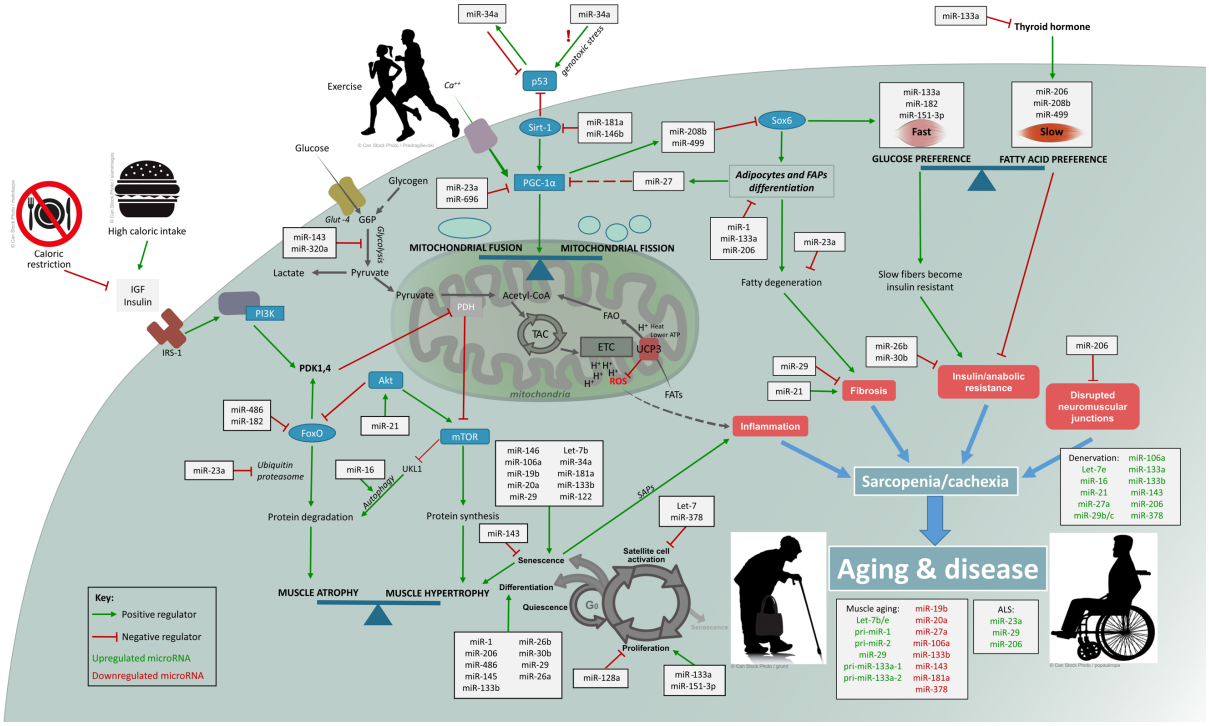

Figure 1. Proposed model of microRNAs regulating muscle homeostasis during aging and disease. Skeletal muscle homeostasis and plasticity depends on the ability of the muscles fibers to use energy efficiently under diverse metabolic demands, this is associated with mitochondrial dynamics. Skeletal muscle insulin resistance, as well as inflammation, fibrosis and disrupted neuromuscular junctions greatly contribute to the loss of muscle mass and strength during ageing and disease. On the molecular levels, some of the key pathways involved in regulation of muscle homeostasis include FoxO and AKT/mTOR, which can be modulated by insulin-like growth factors such as IGF-1, resistance exercise and food intake. Protein degradation and muscle atrophy is driven by FoxO and NFkB pathways under cytokines and chemokines stimuli and trough the ubiquitin-proteasome system. Myostatin, a cytokine member of the TGFβ family, is known to supress muscle growth by inducing FoxO, whereas myostatin inhibitors such as ActRIIA/ActRIIB, follistatin and miR-27a promote muscle hypertrophy. mTOR pathway is also essential for satellite cell activation and *de novo* synthesis of macromolecules needed for cell proliferation. Senescent cells, which are permanently in cell arrest, are metabolically active (inducing mass growth and hypertrophy) and may adopt a pro-inflammatory phenotype (SAPS) contributing to chronic inflammation. Cell proliferation and differentiation are also regulated by physiological concentrations of reactive oxygen species (ROS). ROS is autoregulated by the antioxidant system in response to increased levels of AMPK and PGC-1α.

During ageing, Sirt-1 is unable to deacetylate and activate PGC-1α. Impaired activity of PGC-1α has been shown to correlate with oxidative stress, mitophagy, muscle atrophy as well as a more glycolytic phenotype, which may contribute to slow muscle fibers to become insulin resistant.

Abbreviations: ActRIIA/ActRIIB: dual activin-type II receptor antibody inhibitor to myostatin; Akt: serine/threonine-specific protein kinase (also protein kinase B, PKB); AMPK: 5' AMP-activated protein kinase; ATP: adenosin triphosphate; CAT: catalase; CDK2: cyclin-dependent kinase 2; ETC: electron transport chain; FAO: fatty acid oxidation; FoxO: forkhead box-O; G6P: glucose 6-phosphate; Glut-4: glucose transporter type 4; GPX: glutathione peroxidase; GSH: glutathione; IGF-1: insulin-like growth factor 1; IRS-1: insulin receptor substrate 1; MAPK: mitogen-activated protein kinase; MFF: mitochondrial fission factor; mTOR: mammalian target of rapamycin; mTORC1: mammalian target of rapamycin complex 1 ; mTORC2: mammalian target of rapamycin complex 2; NADPH: nicotinamide adenine dinucleotide phosphate; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; p21: cyclin Dependent Kinase Inhibitor 1A; p27: cyclin Dependent Kinase Inhibitor 1B; p38 MAPK: p38 mitogen-activated protein kinases; p53: tumour protein p53 or TP53; PDH: pyruvate dehydrogenase; PDK: pyruvate dehydrogenase kinase; PDK1: pyruvate dehydrogenase kinase 1; PGC-1α: peroxisome Proliferator-Activated Receptor Gamma Coactivator-1-Alpha; PI3K: phosphoinositide 3-kinase; PIP3: Phosphatidylinositol (3,4,5)-trisphosphate; PTEN: phosphatase and tensin homolog; RB: retinoblastoma tumor supressor; ROS: reactive oxygen species; SAPs: senescence-associate secretory phenotype; Sirt-1: NAD-Dependent Protein Deacetylase Sirtuin-1; SODs: superoxide dismutases; Sox6: SRY (Sex Determining Region Y)-Box 6; TAC: tricarboxylic acid cycle; TGFβ: transforming growth factor beta; TNF-α: tumor necrosis factor alpha; UCP3: mitochondrial uncoupling protein 3; UKL1: uridine kinase-like protein 1.

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