Minireview *Highlight article*

Exosomal microRNAs in cancer-related sarcopenia: Tumor-derived exosomal microRNAs in muscle atrophy

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Impact statement

Cancer-associated sarcopenia is a complex metabolic syndrome marked by muscle mass wasting, which is a serious complication that is a primary contributor to cancer-related mortality. Through the detection of cancer-related low muscle mass, the mortality rate of cancer can be roughly estimated. We can improve the survival rate by maintaining muscle quality. Therefore, in formulating measures to prevent or reduce this muscle-weakening process, significant therapeutic benefits are expected. A special focus is placed on cancer-derived exosomal miRNAs, because they are involved in the communication between muscle cells and tumor cells in cancer-related sarcopenia. Also, it could be used to detect the progression of muscle atrophy. This review is offered to provide basic data for use in the restoration of muscle health in cancer-related sarcopenia, thus promoting future research and treatment strategies.

Abstract

Cancer-associated sarcopenia is a complex metabolic syndrome marked by muscle mass wasting. Muscle wasting is a serious complication that is a primary contributor to cancer-related mortality. The underlying molecular mechanisms of cancer-associated sarcopenia have not been completely described to date. In general, evidence shows that the main pathophysiological alterations in sarcopenia are associated with the degradation of cellular components, an exceptional inflammatory secretome and mitochondrial dysfunction. Importantly, we highlight the prospect that several miRNAs carried by tumor-derived exosomes that have shown the ability to promote inflammatory secretion, activate catabolism, and even participate in the regulation of cellular degradation pathways can be delivered to and exert effects on muscle cells. In this review, we aim to describe the current knowledge about the functions of exosomal miRNAs in the induction of cancer-associated muscle wasting and propose potential treatment strategies.

Keywords: Exosome, miRNA, cancer-associated sarcopenia, muscle atrophy

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Introduction

Sarcopenia occurs when certain muscles are denervated or unable to move. It can also be caused by a systematic response, mainly due to fasting and various diseases. Diseases that manifest this systemic response include septicaemia, acquired immunodeficiency syndrome, renal and heart failure, and an excess of glucocorticoids, such as observed in Cushing's syndrome and trauma. Moreover, more than 80% of cancer patients present with a systemic reaction called cancer-related sarcopenia,[1] which is defined by the European Working Group on Sarcopenia in Older People as a low skeletal muscle mass index with poor handgrip strength, low level of physical performance, and slow gait.[2] The definition is based on a geriatric context but can be used as a reference for cancer. During cancer-related sarcopenia, the accelerated degradation of myofibrillar protein and decreased protein synthesis lead to muscle atrophy. Evidence from research indicates that sarcopenia may be related to factors such as synthetic resistance and endothelial dysfunction.[3] Moreover, clinical studies have indicated that, through the detection of cancer-related low muscle mass, the mortality rate of cancer can be roughly estimated.[4] According to these outcomes, we can improve the survival rate by maintaining muscle quality.[1] Therefore, in formulating measures to prevent or reduce this muscle-weakening process, significant therapeutic benefits are

expected, especially for a series of different clinical conditions. In general, nutrition supplementation and physical exercise, including aerobic exercise and resistance exercise, can maintain the quality of collateral bone and are the main key interventions to implement.[5] However, the mechanism and more efficient therapeutic methods should be further studied.

Exosomes are membrane-bound vesicles. Exosomes and their contents are released into the extracellular environment upon the fusion of late endosomes with the plasma membrane. Proteins, RNA, DNA, and lipids are all components of exosomes.[6] They can be transported by paracrine action at systemic levels, participating in cell communication and mediating cancer development.[7–10] In particular, microRNAs (miRNAs) in exosomes can promote tumor seeding, metastasis and drug resistance through cell–cell communication in the tumor microenvironment.[11] Noncoding RNA molecules consisting of 19–24 nucleotides constitute a subfamily of miRNAs. Their functions in regulating gene expression is mainly realized by their roles in degrading messenger RNA (mRNA) and thus inhibiting protein translation.[4] Changes in the miRNA expression profile can be observed in the deteriorating muscle of injury-inducing diseases.[4] These changes are possibly caused by miRNA exosomes participation in inflammatory signal transduction and activation of muscle catabolism.[4]

In this review, the molecular mechanism of sarcopenia pathophysiology is introduced. A special focus is placed on cancer-derived exosomal miRNAs, which will capture the attention of researchers because they constitute a new effective strategy for the study of sarcopenia. This review is offered to provide basic data for use in the restoration of muscle health in cancer-related sarcopenia, thus promoting future research and treatment strategies.

Pathophysiological mechanisms of cancer-related sarcopenia

Synthesis and degradation of cellular components

Decreased protein synthesis, increased degradation or creation of a relative imbalance between the anabolism and catabolism lead to the loss of skeletal muscle quality.[1] All intracellular proteolytic pathways operating in skeletal muscle (proteasome, lysosomes, caspases, and calpains) have been shown to be activated above physiological levels in cancer-related muscle atrophy.[12]

The ubiquitin-proteasome system (UPS) is the main regulatory mechanism by which skeletal muscle atrophy consumes ATP. It mediates the degradation of target proteins recognized upon ubiquitin conjugation.[5,13,14] In experimental models of cancer-related muscle atrophy and human studies involving cancer patients, the activation of the UPS in skeletal muscle has been reported and confirmed. The average weight loss in gastric cancer (GC) patients was 5.2%, and the UPS activity in GC patients was higher than it was in the control group patients. The tumor stage and weight loss contributed to the increased activation of the UPS.[15] The function of the UPS is mainly regulated by ubiquitin activator (E1), ubiquitin binding enzyme (E2) and, the most important, ubiquitin ligase (E3). E3 determines the specificity of this regulatory mechanism.[16] Increased mRNA levels of muscle atrophy F-box protein (*MAFbx*, also known as *FBXO32* or *Atrogin-1*), muscle RING-finger 1 (*MuRF1*, also known as *TRIM63*), and muscle-specific E3s, termed "atrogenes", were reported in tumor experimental models.[16,17] Two kinds of proteins are potential targets of *MuRF1*: proteins involved in ATP production and myofibrillar proteins. First, the proteasomes and transcriptomes of *MuRF1*-transgenic mice and wild-type (WT) mice were compared. The results showed that *MuRF1* may play a role in metabolism regulation because there were significant differences in the enzymes involved in ATP production, especially enzymes involved in glycolysis.[18] Second, C2C12 myoblast cells were infected with a retrovirus encoding human MuRF1 protein. The infected cells were differentiated into myotubes for three days. MuRF1 was found to be capable of immunoprecipitating myosin heavy chain (MYH), whereas significantly less MYH was found in uninfected C2C12 myotubes.[19] In addition, we also concluded that the final ubiquitination and degradation of MyHC upon muscle atrophy is dependent on MuRF1, not on actin or other fibres.[20]

The most widely recognized target of *FBXO32* in skeletal muscle is MyoD, a myogenic regulatory factor.[21] The direct connection between MyoD and *FBXO32* is strongly supported during the development of C2C12 myotube cells. The UPS mediates the degradation of MyoD, and *in vitro* experiments revealed that *FBXO32* interacts with MyoD, the ubiquitination of which is mediated via a lysine-dependent pathway.[21]

In addition to the UPS, the autophagy-lysosome system (ALS) plays an important role in cancer-related sarcopenia. Autophagy is a conservative self-degradation system that is of great significance for maintaining cell homeostasis under stress *in vivo*.[5] In cancer-related muscle atrophy, autophagy is activated and involved in muscle wasting.[22] The latest research results show that the aggravation of muscle atrophy symptoms caused by cancer is due to autophagic regulation of overexpressed TP53INP2/DOR protein.[23] The maintenance of ubiquitin protein degradation depends on TP53INP2 activating basal autophagy, which directly interacts with skeletal muscle LC3. In particular, the ALS is also interconnected with the UPS. Compared with that in WT tumor-bearing mice, the overexpression of TP53INP2 leads to a further increase in *FBXO32* and *MuRF1*.[23,24] Moreover, the forkhead box protein O (FoxO) family can directly upregulate *MuRF1*.[2] In addition, muscle atrophy caused by FoxO3 overexpression requires autophagy *in vivo*, which can be partially prevented by LC3 knockdown.[25]

Alternatively, the upregulation of sarcopenia-related molecules can be inhibited by protein kinase B (Akt) signaling.[15] It has been reported that, insulin resistance was an early event during cancer-related muscle atrophy. Insulin-like growth factor-1 (IGF-1)/phosphatidylinositol-3 kinase (PI3K)/Akt is a central signaling in the insulin signaling cascade,[1,26,27] which promoted skeletal muscle anabolism.[28–31] First, IGF-1 is transphosphorylated, and the docking site of insulin receptor substrate-1 (IRS-1) is formed after IGF-1 binds to its transmembrane tyrosine kinase

receptor.[28] Then, IGF-1 induces the phosphorylation of IRS-1, and the PI3K/Akt pathway is activated, which inhibits FoxO and glycogen synthase kinase-3 (GSK3) and prevents the activation of myostatin in mothers against decapentaplegic homologue (SMAD)2 by myostatin. Therefore, protein synthesis is increased.[29,32] In addition, several E3 ubiquitin-protein ligases can mediate the downstream signaling through the IGF-1/PI3K/Akt pathway. For instance, the E3 ligase MDM2 is able to promote monoubiquitylation and polyubiquitylation upon nuclear sorting or the degradation of FoxO. The regulation mediated by MDM2 varies according to the phosphorylation state of FoxO proteins. Therefore, the ubiquitination and degradation mediated by MDM2 are promoted by Akt phosphorylation of FoxO. In contrast, MDM2 monoubiquitylates non-phosphorylated FoxO in oxidative stress conditions, thereby promoting the subsequent nuclear localization of FoxO.[33,34] However, the level of IGF is not always abnormal. Insulin resistance (IR) occurs in colorectal cancer, non-small cell lung cancer (NSCLC), gastrointestinal cancer, and mixed malignant tumors.[35] IR can inhibit protein synthesis in many cancer models. Recent genetic studies have identified the tumor secretion factor called ImpL2, which is an insulin growth factor-binding protein homologue that participates in the interaction between tumor and muscle cells and results in muscle atrophy.[36] Another potential mechanism of protein degradation mainly involves the interaction between IR and UPS. IR contributes to the decrease in phosphorylation of PI3K and Akt phosphorylation, which further inhibits the release of FoxO and caspase-3, which enhances the hydrolysis of proteins.[37]

Collectively, the UPS and ALS are the main mechanisms that regulate protein degradation, while IGF-1 signaling dominates a protein synthesis process. The activation of the UPS and ALS with simultaneous inhibition of IGF-1 signaling leads to muscle wasting in cancer.

Secreted factors

Many secreted factors can mediate the process of sarcopenia, such as pro-inflammatory cytokines, transforming growth factor-β (TGF-β) family members, and some hormones. These secreted factors increase in cancer-related sarcopenia which all promote muscle atrophy to a certain extent.[38–41]

Inflammatory cytokines play important roles *in vivo* in the pathogenesis of cancer-related muscle atrophy. In tumor patients, pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6 and interferon-γ, increase,[40] and the expression of anti-inflammatory cytokines, such as IL-10, decreases,[42] which promotes the expression of *FBXO32* and *MuRF1* through the production of reactive oxygen species (ROS) and the activation of FoxO protein.[43–45] For example, TNF-α has been shown to stimulate ROS production in mitochondria by activating NADPH oxidase and xanthine oxidase, leading to high H_2O_2 levels.[46] H_2O_2 can activate both NF-κB and SMAD3, on the one hand enhancing the inflammatory response and, on the other hand, promoting catabolism of muscle protein by inducing the transcription genes encoding muscle-specific ubiquitin ligase.[47] In addition, TNF-α mediates mitogen-activated protein kinase p38 signaling and FoxO transcription, which can increase *FBXO32* expression.[48,49] Alternatively, pro-inflammatory cytokines mediate protein degradation through the ALS. IL-6 plays a very important role in the regulation of the ALS. Increasing IL-6 was found in the serum of patients with muscle wasting. Incubating C2C12 myoblasts with the IL-6 collected from these patients resulted in elevated p62 and LC3 levels.[50] The latest experimental results showed that the inhibition of mTOR activation by high circulating IL-6 levels led to the generation of cancer-related muscle atrophy in Apc(min/+) mice.[51] Previous research results also showed that AMPK phosphorylation in skeletal muscle and autophagy signaling were inhibited by an IL-6 receptor antibody in muscle atrophy Min mice.[52] In addition to promoting protein degradation, pro-inflammatory cytokines also retard protein synthesis. IGF-1 signaling can be downregulated by these cytokines. For example, the increase in circulating TNF-α and IL-1, which inhibit the Akt signal transduction pathway, is the main cause of muscle atrophy induced by lipopolysaccharide.[53,54] In fact, when TNF-α phosphorylates a serine of IRS-1, the recruitment of IRS-1 to the insulin/IGF-1 receptor is blocked. Through the direct interaction between the IKK complex and IRS-1, TNF-α directly affects insulin/IGF-1 signaling. In addition, TNF-α-induced c-Jun N-terminal kinase (JNK) activation may be involved in the JNK inhibitor-induced reversal of the downregulation of IGF-1 signaling induced by cytokines.[55]

Another secreted factor involved in muscle wasting is myostatin, which is mainly expressed in skeletal muscle and belongs to the TGF-β family.[56] Myostatin can promote cancer-associated muscle atrophy. The binding of myostatin to activin receptor 2B (ACTRIIB) on muscle membranes leads to the phosphorylation of SMAD2 and SMAD3,[56,57] thus inducing muscle protein degradation by the UPS in a FOXO-dependent manner as mediated by Akt and inducing decreased muscle protein synthesis by inhibiting Akt.[58–60] Specifically, treatment with sACTRIIB, an inhibitor of myostatin-mediated SMAD2/3 signal transduction, successfully reversed the weight loss in C26 tumor-implanted mice, despite more than 10% of mouse body weight having been previously lost.[59] After myostatin treatment, muscle atrophy was induced, and the *FBXO32* and *MuRF1* levels increased,[60] but this upregulation was not observed in SMAD2/3 doubly deficient mice.[61] Furthermore, blockade of the UPS prevented myostatin-mediated muscle wasting. Treatment with myostatin resulted in increased total FoxO1 and reduced p-FoxO1 and p-Akt levels, which suggested that myostatin is able to activate FoxO1 by inhibiting the IGF/Akt signaling pathway.[60]

Moreover, some hormones participate in the process of cancer-related sarcopenia. Metabolic changes in protein and glucose in skeletal muscle are regulated by glucocorticoids.[39] Glucocorticoid treatment increases FoxO-dependent transcription of E3 ubiquitin ligases, thus promoting protein catabolism and inhibiting protein synthesis, which contributes to muscle atrophy.[62] Increased glucocorticoid levels were also observed in cancer patients.[63]

Cancer-related muscle wasting is mediated by complete glucocorticoid signals in skeletal muscle.[64] Dexamethasone was administered to mice whose target of glucocorticoid receptor was deleted in muscle (mGRKO mice). In these mice, muscle atrophy was dramatically attenuated, and their muscle mass loss was reduced to 65% compared with that of the control group. In addition, the expression of *FBXO32*, *MuRF1* and FoxO1 induced by dexamethasone is also blocked. Furthermore, LPS significantly increased *FBXO32*, *MuRF1*, and FoxO1 levels in the control mice but this effect was blocked in the mGRKO mice. A cancer cachexia model was established in the mGRKO mice and control mice. Compared with the mGRKO mice, the muscle mass of the control group mice was decreased significantly due to tumor growth.[64] In addition, as a member of the parathyroid hormone family, parathyroid hormone-related protein (PTHrP) is expressed in many cancers and contributes to their progression and metastasis. Moreover, it is involved in muscle wasting. PTHrP can increase the expression of atrophy-related genes in tumor-bearing mice,[65] and anti-PTHrP antibody therapy can reverse the effect of skeletal muscle atrophy. However, the best therapeutic effect of antibody therapy is the prevention of adipose tissue loss and energy consumption in the body. PTHrP cannot directly affect primary muscle cells, but it is likely to promote muscle atrophy by promoting the interaction between adipose and muscle tissues.[65,66] Furthermore, patients with high circulation PTHrP have higher resting energy expenditure and lower lean mass.[67] Therefore, PTHrP is used to predict weight loss in cancer patients, which indicates that it may also be a biomarker of cancer-related muscle atrophy.[65] In addition, leptin can promote muscle wasting. Leptin not only can regulate the fat quality of mammals but can also regulate the food intake and energy balance of human beings. Recent experimental results suggested that overfeeding is likely to lead to weight loss in fish with hepatocellular carcinoma (HCC). Moreover, leptin was observed to be increased after $kras^{G12V}$ induction in oncogenic hepatocytes, and excessive feeding enhanced this increase. It was reported that knocking out the leptin receptor gene reduced muscle atrophy in HCC. When the downstream signaling pathways of leptin, including JAK2/STAT3, were inhibited, HCC-induced muscle atrophy was alleviated.[41] Overall, cancer-related muscle atrophy is caused by the secretion of various potential mediators directly or indirectly induced by tumors.

Mitochondrial dysfunction

An imbalance of energy in the body can cause the decrease of muscle mass. Mitochondria are the centers of energy metabolism in cells, and their dysfunction contributes to cancer-associated sarcopenia. The number of mitochondria decreases in tumor cells. Additionally, the shape of these organelles changes, and the mitochondrial matrix is diluted. In addition, to a certain extent, mitochondrial function is also damaged.[68]

Mitochondrial function is maintained by continuous fission and fusion, a process that removes poorly functional content and mixes the remaining mitochondrial content to enhance oxidative capacity.[69] Data from the GEO database suggest that fission-related genes, *Dnm1l* and *Oma1*, and fusion-related genes, *Mfn2* and *Opa1*, are downregulated during cancer-related muscle atrophy. Mitochondrial fusion and fission rates are also decreased, eventually changing the shape of mitochondria, which has adverse effects on the quality control of mitochondria.[70] Transmission electron microscopy images revealed that the area of mitochondria in the skeletal muscle affected by cancer-related muscle atrophy is relatively large.[71] In addition, in the process of cancer-related muscle atrophy, swelling mitochondria, vesicle-like structures, and disrupted triads were observed.[72–74] In addition, mitophagy is upregulated during mitochondrial damage. Mitophagy, a process of selectively degrading impaired mitochondria, is vital for a healthy mitochondrial population. In cachectic muscle tissue, the expression of Sqstm1 and ubiquitin, which label mitochondria for degradation, are upregulated at the protein and gene levels.[75] The upregulation of mitophagy impairs mitochondrial function. In tumor-bearing mice, the mitochondrial respiration of the extensor digitorum longus muscle fibre bundle was seriously damaged. Respiratory complex I and II were decreased in mice with muscle atrophy. Further study demonstrated an increase in BNIP3, which suggested damaged mitochondria. Consistent with increased bulk autophagy activated in atrophic muscle, LC3B-II was increased in the cytosol, especially in the mitochondrial fraction.[23]

Furthermore, mitochondrial function is impaired during muscle atrophy. Mitochondrial uncoupling proteins (UCPs) localize to the mitochondrial membrane, which promotes thermogenesis but not ATP generation by mediating proton leakage and uncoupling respiration.[76] Mitochondrial ATP synthesis in tumor-bearing mice is reduced, and respiratory uncoupling is also observed, which suggests that UCPs play a role in cancer-related muscle atrophy.[77–79] UCP-1 is expressed in adipocytes. It is essential for the "browning" of white adipose tissue (WAT), which indicates a switch from WAT to thermogenic beige adipocytes that occurs during cancer-related muscle atrophy.[80] Compared with cancer patients without muscle wasting, cancer patients with muscle atrophy have higher expression of UCP1 in adipose tissue, which may lead to muscle atrophy and further thermogenesis.[80] By secreting PTHrP, which can induce UCP1, the tumor can directly activate the heat production of the beige cells. UCP1 stimulates thermogenesis in muscle atrophy mice at the expense of ATP synthesis, thus increasing fat mobilization and energy consumption.[65] On the other hand, UCP-2 and UCP-3 promote muscle atrophy. The expression of UCP2 and UCP3 genes in the skeletal muscle of muscle atrophy animals is relatively high.[81] In addition, UCP2 converts oxidative phosphorylation into glycolysis.[82] UCP2 can stimulate the expression of the glucose transporter GLUT1 and pyruvate kinase isoform M2 mRNA. When UCP2 is inhibited, the components of mitochondrial oxygen consumption, such as complex I, complex IV and complex V, are downregulated.[82] In addition, UCP3 contributes to weight loss. The mRNA level of UCP3 in 12 patients with gastrointestinal adenocarcinoma was

measured. The average level of UCP3 in the experimental group of patients with weight loss was more than five-fold higher than that in the control group.[83] Mice that overexpressed UCP3 presumably weighed less than the wild-type mice, despite being overfed. Moreover, fasting plasma glucose levels and insulin levels were decreased, and glucose clearance was increased after an oral glucose load was administered to transgenic mice, which suggested that UCP3 modulated the metabolic rate and glucose homeostasis.[84]

On the other hand, in general, for maintaining muscle quality, mitochondrial function and oxidation ability need to ensured.[85] Oxidative stress reactions can be caused by the accumulation reactive oxygen or nitrogen species (ROS/RNS). When ROS accumulation exceeds the adaptation capacity of cells, several molecular pathways are activated, eventually leading to muscle atrophy. High oxidative stress can directly or indirectly modulate both the protein synthesis machinery and degradation pathways. Indeed, ROS are known modulators of the PI3K/Akt/mTORCl pathway, the main intracellular regulator of protein synthesis.[86] For protein degradation machinery function, excess ROS can alter calcium homeostasis, which in turn activates the cysteine protease calpain, leading to myofilament release.[87] In parallel, an imbalance in calcium levels activates abnormal NF-KB expression, simultaneously activating protein degradation.[88] In addition to the calpain system, severe muscle wasting is generally associated with the hyperactivation of a degradation system that entails the deposition of a large number of proteins and organelles, namely, the UPS or ALS. For the former, a direct link with oxidative stress has been suggested by *in vitro* observations of C2C12 myotubes, where exposure to H_2O_2 results in increased enzymatic activity and expression of proteasome components.[89] In addition, a recent study has shown that diaphragm atrophy during mechanical ventilation depends on ROS-induced proteasome and autophagy activation.[90] In this regard, crosstalk between the components of oxidative stress and autophagy has been previously suggested by observations suggesting that blocking mitochondrial ROS production using antioxidants in rats attenuated both increased autophagy and muscle wasting.[91] In summary, mitochondria are impaired during cancer-related sarcopenia, and the impaired mitochondria further exacerbate the muscle atrophy.

Cancer-derived exosomal microRNAs in sarcopenia

The roles of exosomes in cancer-related muscle atrophy have attracted considerable attention because exosomes promote the communication of various tissues by paracrine and endocrine mechanisms by carrying proteins and miRNAs. As muscle atrophy is a systemic syndrome, exosomes and the cargo they carry are likely to mediate the process. Exosomal miRNAs directly or indirectly participate in the wasting of muscle by regulating protein degradation, inflammation, and energy metabolism. The conclusions from these studies are shown in Table 1.

miRNA-126

The signals of tumor cell proliferation, migration, invasion, and survival can be controlled by *miR-126* and *miR-126**, which can inhibit the progression of cancer to a certain extent. In contrast, *miR-126* and *miR-126** also influence and support tumor progression by promoting vascular growth and mediating inflammation.[92] The fact that *miR-126* can be secreted into the environment around the tumor has been proven.[93,94] Some cancers can be detected by the plasma concentration of *miR-126*, which serves as a biomarker.[94,95] In general, the content of serum *miR-126* is decreased, but the content of *miR-126* in exosomes is increased.[95] The increase in *miR-126* in exosomes *in vitro* is caused by oxidative stress and glucose deficiency.[96–98] Exosomal *miR-126* can promote muscle atrophy. Exogenous *miR-126* is a significant promotor of the transcriptional response to decreased lean mass.[99] An *miR-126* antisense oligonucleotide sequence and miR-126 mimic were transfected into C2C12 myocardial cells. Inhibition of miR-126 decreased FBXO32 nearly six-fold. The level of IRS-1 was reduced due to the overexpression of *miR-126*, while the expression of *MuRF1* and *FBXO32* was increased.[99] Mechanistically, experimental evidence

Table 1. microRNAs involved in cancer-related muscle atrophy.

MicroRNA	Target	Promote/inhibit muscle atrophy	Main function	References
miR-126	IRS-1	Promote	-Inhibit IRS-1 -Increase the expression of atrogenes -Promote glycolysis -Inhibit mitochondrial respiration	Tomasetti *et al.*[100] Rivas *et al.*[99] Tomasetti *et al.*.[96]
miR-155	IGF-1	Unclear	-Inhibit WAT browning -Stimulate muscle cells apoptosis	Chen *et al.*[106] Shen *et al.*[107]
miR-21 & miR-29	TLR-7/8	Promote	-Induce cell death -Promote inflammation -Contribute to nuclear localization of FoxO	He *et al.*[114] Fabbri *et al.*[116] Borja-Gonzalez *et al.*[117]
miR-182	FoxO3	Inhibit	-Inhibit FoxO3 -Maintain glucose homeostasis, enhance glucose oxidation	Hudson *et al.*[124] Zhang *et al.*[125]

FoxO: forkhead box protein O; IGF-1: insulin-like growth factor-1; IRS-1: insulin receptor substrate-1; TLR: toll-like receptor; WAT: white adipose tissue.

indicated that *miR-126* participates in protein degradation mainly by targeting IRS-1.[96,100,101] LC3II accumulation was observed after IRS-1 overexpression, in contrast to the inhibition of IRS-1 leading to reduced LC3II levels in the *miR-126*-transfected cells. IRS-1 lacking the *miR-126* binding site was used to further prove that *miR-126*-induced autophagy is partially IRS-1-dependent.[100] Additionally, *miRNA-126* inhibits IRS-1, subsequently suppressing Akt activation. Akt activation by IGF-1 was significantly increased 15-fold in miR-126 inhibitor-transfected cells compared with vehicle. Phosphorylation of FoxO1, an Akt target and myogenic regulator, was increased dramatically as well. The FoxO1 transcription factor was isolated in the cytoplasm after being phosphorylated by Akt. As a result of nuclear localization, FoxO1 caused increased expression of gluconeogenesis genes and protein degradation.[96,99] In addition, *miR-126* also participates in glucose metabolism. Glucose transporter-4, the main glucose carrier, was upregulated in *miR-126*-transfected cells.[100] Ectopic *miR-126* increased ATP and lactate levels, which were inhibited by 2-deoxyglucose.[96] Finally, *miR-126* is able to inhibit mitochondrial function. *miR-126*-transfected cells were subjected to rotenone exposure. Mitochondrial DNA (mtDNA) depletion, $CoCl_2$ exposure, and mitochondrial reducing activity (MRA) were evaluated. Rotenone and mtDNA depletion increased the MRA, which was further increased by *miR-126*. The MRA was decreased in response to $CoCl_2$ and was slightly increased by *miR-126*. Additionally, the MRA reduced the ROS formation induced by rotenone. ROS are generated in response to $CoCl_2$ and mainly consisted of peroxide-related species, but not superoxide, and their levels were reduced in *miR126*-transfected cells.[96] Overall, *miR-126* promotes muscle atrophy by reducing mitochondrial respiration, promoting glycolysis, and blocking cytosolic sequestration of FoxO1.

miRNA-155

miR-155-3 can promote cell proliferation and tumorigenesis and inhibit cell apoptosis. It is upregulated in breast cancer.[102] Exosomal *miR-155* participates in tumor progression. *MiR-155* can be carried by exosomes and promote angiogenesis in GC by inhibiting FoxO3a, which plays a positive role in GC progression.[103] The increased level of encapsulated exosomal *miR-155* has also been found in chronic lymphocytic leukaemia (CLL) plasma and is further increased upon B cell receptor activation.[104] Recent studies revealed that tumor-derived exosomal *miR-155* increased metabolite release, which promoted catabolism in adipocytes and muscle cells.[105] *miR-155* in exosomes derived from breast cancer cell modulates the process of WAT browning. The increased expression of UCP1 promoted by the *miR-155* sponge was much higher than that induced by norepinephrine. UCP1 expression was more than 20-fold higher when endogenous *miR-155* was inhibited. Additionally, the expression of UCP1 was decreased in LV*miR-155*-transfected cells.[106] Moreover, *miR-155* can stimulate the apoptosis of muscle cells by reducing IGF-1 levels. In colonic smooth muscle cells (SMCs), *miR-155* downregulated the level of IGF-1 at the post-transcriptional level. Cell viability increased after IGF-1 was overexpressed. Overexpressing *MiR-155* and IGF-1 at the same time reduced cell viability and the apoptosis rate. Furthermore, the overexpression of *miR-155* not only increased the apoptosis rate of colon SMCs but also decreased the thickness of the colon smooth muscle tissue. In contrast, the knockdown of *miR-155* induced the opposite effect.[107] These results indicate that *miR-155* aggravates colonic dysmotility. Therefore, the effect of *miR-155* on muscle wasting remains unclear because it inhibits WAT browning, thus decreasing thermogenic action, but induces muscle cell apoptosis.

miRNA-21 and miRNA-29

miR-21 is highly expressed in breast cancer and is related to lymph node metastases, clinical stage, and differentiation.[108] *miR-21* is associated with tumor proliferation. It is able to reduce the cell death of NSCLC cells and hepatoma cells by downregulating the PI3K/Akt pathway.[109,110] On the other hand, *miR-29* negatively modulates the pathogenesis of osteosarcoma.[111] It is able to promote the apoptosis of cholangiocarcinoma and HCC cells.[112,113]

Exosomal *miR-21* is increased in lung and pancreatic cancer.[4,114] The upregulation of exosomal *miR-29a* was found in the plasma of patients with CLL and lung cancer.[104,115] Exosomes containing *miR-21* were extracted from Lewis lung carcinoma and added to C2C12 myoblasts, which induced their death.[114] Specifically, *miR-21* and *miR-29a* were encapsulated in exosomes in the lung cancer cells, and acted as ligands containing a GU motif known to activate ssRNA-binding TLRs and selectively activated toll-like receptor 7 (TLR-7) in rat cells and toll-like receptor 8 (TLR-8) in human myoblasts.[114,116] The *miR-21*-induced apoptosis of TLR-$7^{+/+}$ primary myoblasts was weaker in TLR-$7^{-/-}$ cells.[114] Myoblasts were treated with JNK inhibitor with or without exosomal *miR-21*. In the presence of *miR-21*, the JNK inhibition can suppress myoblast apoptosis.[4,114] In addition, the TLR-7/8–MyD88–NF-κB pathway was activated by *miR-21* and *miR-29*. Downstream signaling molecules, such as TNF-α and IL-6, were upregulated to promote protein degradation and decrease protein synthesis. In addition, an increase in *miR-21* contributed to the translocation of *FoxO3* to the nucleus. An increase in cell stress and apoptosis was related to this translocation. In *miR-21*-treated myoblasts, the mRNA level of the cell DNA damage protein Gadd45 was upregulated compared with the level in the untreated cells. The expression of the anti-apoptosis gene *Bcl-2* and mitochondrial marker Nd-1 was also initially upregulated when *miR-21* was inhibited.[117] In summary, *miR-21* and *miR-29* can promote cancer-associated sarcopenia, mainly by inducing cell apoptosis via TLR7/8 signaling.

miRNA-182

The high expression of *miR-182-5p* in tumor tissues and the tumor microenvironment is related to poor prognosis and early recurrence.[118–121] The exosomes containing *miR-182* alleviated muscle atrophy. According to research, *miR-182-5p* was detected in the exosomes of glioblastoma, and

the expression of *miR-182-5p* was found to be significantly upregulated under hypoxic conditions.[122] The exosomes containing *miR-182* caused less muscle atrophy. After C2C12 myotubes were treated with a FoxO3a activator, the intracellular *miR-182* content was significantly reduced, by 44%, indicating that the *miR-182* content was reduced during muscle atrophy. Similarly, it was proven that *miR-182* targets the *FoxO3* 3′UTR directly.[123,124] *miR-182*-transfected muscle cells exhibited downregulated *FoxO3* at the mRNA and protein levels. Additionally, multiple glucocorticoid-upregulated genes targeted by FoxO3, including *FBXO32*, cathepsin L. and autophagy-related proteins, were inhibited.[124] In addition, *miR-182* modulated glucose utilization in muscle. Knocking out *miR-182* led to impaired glucose tolerance in mice, which suggested that normal glucose homeostasis requires *miR-182*. Furthermore, the mRNA and protein levels of pyruvate dehydrogenase kinase 4 (PDK4) in C2C12 myotubes were also decreased due to the overexpression of *miR-182*. To verify whether the effect of *miR-182* on glucose metabolism is FoxO1/PDK4-dependent, a plasmid carrying FoxO1 lacking its *miR-182* binding site was introduced into C2C12 myotubes transfected with an *miR-182* inhibitor. It was observed that the inhibition of PDK4 at the gene and protein levels by *miR-182* was weakened. Additionally, glucose oxidation was measured. The experimental results showed that glucose oxidation was inhibited when *miR-182* was knocked out, while the overexpression of *miR-182* led to the opposite outcome, suggesting that *miR-182* regulated glucose oxidation by modulating the expression of the pyruvate dehydrogenase complex via PDK4.[125] In summary, *miR-182* attenuates muscle wasting by directly targeting FoxO3a and maintaining glucose homeostasis.

Conclusions

In summary, current studies have shown that the degradation of cell components, an exceptional inflammatory secretome and mitochondrial dysfunction are closely associated with muscle wasting (Figure 1). Exosomes, as mediators of the crosstalk between systemic organs, can regulate the function of skeletal muscle. They may have potential as a research strategy for sarcopenia.

In terms of the mechanism of cancer-related sarcopenia, several outstanding issues remain. Due to the lack of reliable protein expression data, the understanding of *MuRF1* and *FBXO32* has led to controversial outcomes. Although changes in the mRNA of sarcopenia-related genes have been explored, neither the kinetics of protein translation nor the disposal of their ligases have been clarified. In addition, secreted factors in the tumor microenvironment are abundant. Previous studies have discussed their separate effects on muscle wasting, but the interaction among them needs further exploration. Alternatively, the main changes in muscle mitochondria in tumor state are altered mitochondrial morphology, decreased ATP synthesis, and enhanced respiratory uncoupling. Therefore, in the

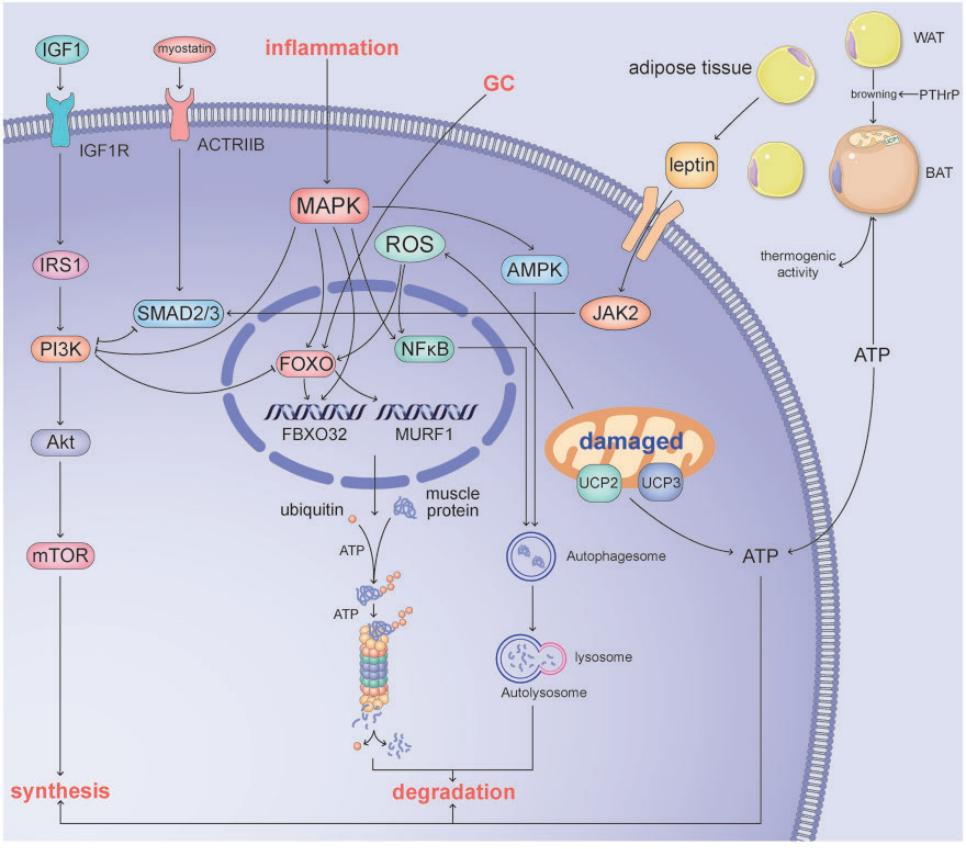

Figure 1. Mechanisms involved in cancer-related muscle atrophy.

future, we should conduct in-depth research on the effects of these factors causing the mitochondrial dysfunction, such as different cytokines and the origin of oxidative stress.

Exosomes containing miRNAs can promote communication among various organs. However, a systematic analysis of the effects of multiple miRNAs carried by exosomes is missing. A comprehensive assessment of circulating exosomal miRNAs may enable prediction of muscle atrophy. Furthermore, the expression of atrogenes and the levels of autophagy, the secretome and mitochondrial functions can all be included in the prediction model. Moreover, it is essential to determine the physiologically relevant amounts of secreted miRNAs required for muscle atrophy.

To improve the condition of patients with muscle atrophy and tumors, diverse strategies have been developed. Among these approaches, exosomes containing miRNAs are potential new targets. It has been reported that physical exercise can induce the rapid release of exosomal miRNAs that are associated with muscle growth.[5] Exercise likely attenuates hypoxia, thereby modulating the levels of miRNAs in exosomes. In addition to inhibiting the release of exosomes from tumors or suppressing the level of atrophy-related miRNAs in exosomes, inhibiting the target of exosomal miRNAs or blocking the intake of exosomes by muscle cells may be a potential therapy. Furthermore, engineered exosomes can deliver miRNAs or drugs that negatively regulate muscle atrophy to muscle cells. Overall, exosomal miRNAs can be important players in the modulation of cancer-associated sarcopenia. The underlying mechanism should be examined more thoroughly in the future, as these efforts will lead to new therapies.

AUTHORS' CONTRIBUTIONS

All authors participated in the design, interpretation of the studies, analysis of the data, and review of the manuscript; QW and CL conceived the review. CL drafted manuscript. SS and SS were responsible for editing and submission. ZL, ZW, YT, and CC are responsible for the revision.

DECLARATION OF CONFLICTING INTERESTS

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