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Review

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Exosome-mediated regulatory mechanisms in skeletal muscle: a narrative review

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Abstract: Skeletal muscle plays a paramount role in physical activity, metabolism, and energy balance, while its homeostasis is being challenged by multiple unfavorable factors such as injury, aging, or obesity. Exosomes, a subset of extracellular vesicles, are now recognized as essential mediators of intercellular communication, holding great clinical potential in the treatment of skeletal muscle diseases. Herein, we outline the recent research progress in exosomal isolation, characterization, and mechanism of action, and emphatically discuss current advances in exosomes derived from multiple organs and tissues, and engineered exosomes regarding the regulation of physiological and pathological development of skeletal muscle. These remarkable advances expand our understanding of myogenesis and muscle diseases. Meanwhile, the engineered exosome, as an endogenous nanocarrier combined with advanced design methodologies of biomolecules, will help to open up innovative therapeutic perspectives for the treatment of muscle diseases.

Key words: Exosome; Skeletal muscle; Muscle atrophy; Insulin resistance

1 Introduction

Skeletal muscle develops from muscle progenitor cells on the side of the paraxial mesoderm. Progenitor cells undergo terminal differentiation to form mononuclear myoblasts (Zeschnigk et al., 1995), which further fuse into myotubes (Steinacker et al., 2000) and ultimately generate biofunctional muscle tissue. Skeletal muscle is a highly dynamic and plastic organ that requires continuous surveillance to maintain biofunctional integrity in response to the demands of movement and metabolism. The biofunctional integrity of skeletal muscle is coordinated by complex regulatory circuits; however, multiple disadvantages, such as injury, aging, inactivity, and the current obesity epidemic, have disrupted this regulatory network and resulted in various skeletal muscle diseases (Wu and Ballantyne, 2017; Larsson et al., 2019).

Early work on extracellular vesicles (EVs) has deepened our understanding of the interactions between organs and tissues. EVs are particles composed of lipid bilayers, and preliminarily classified into exosome, microvesicle, apoptotic body, and oncosome according to their biogenesis, release, and particle size. The diameters of EVs range from 20–30 nm (exomere, 35 nm) (Zhang et al., 2018) to 10 μm (large oncosome, 1–10 μm) (Minciacchi et al., 2015), but most of them are less than 200 nm. Recent studies illustrated that almost all living cells transport specific combinations of proteins, nucleic acids, and lipids between donors and recipients via exosomes, thereby mediating the genetic exchanges and leading to the reprogramming of recipients (Torralba et al., 2018; Asare-Werehene et al., 2020; Chen CH et al., 2021; He et al., 2021; Xu et al., 2022). Convincing evidence has indicated that exosomes play vital roles in the physiology and pathology of skeletal muscle, suggesting the possibility of a novel strategy to ameliorate skeletal muscle dysfunction.

Herein, we review the current progress in exosomal isolation, characterization and mechanism of action. We further comprehensively discuss the physiological and pathological regulatory mechanisms mediated by diverse organs- and tissues-derived exosomes, as well as engineered exosomes, in skeletal muscle. The remarkable advances made in recent years expand our understanding of myogenesis and muscle diseases.

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Moreover, they clearly help to elucidate exosome-mediated regulatory pathways in the physiology and pathology of skeletal muscle, and reveal innovative therapeutic perspectives in protecting against muscle diseases. Meanwhile, the engineering exosome, as an endogenous nanocarrier combined with advanced design methodology of biomolecules, further highlights the clinically translational potential of exosomes.

2 Overview of exosomes

The exosome is a pivotal subtype of EVs originating from endosome-derived multivesicular bodies (Kalluri and LeBleu, 2020; Gurunathan et al., 2021). It was first reported by Pan and Johnstone (1983) and named as "exosome" by Johnstone et al. (1987). It is known to play essential roles in cellular communication by transferring bioactive cargos, while two major technical hindrances limit the relevant research. Firstly, these are challenges in simplifying the isolation method and improving the exosome yield, and secondly, it is difficult to effectively distinguish exosomes from other EV subtypes. In this section, we review the current research progress in exosomal isolation, characterization and mechanism of action.

2.1 Exosomal isolation

The isolation of exosomes is the first step in related research. Considering that a single isolation method applicable to different physicochemical and biochemical sources would be impractical, multiple methods have been established to extract exosomes from various sources. The commonly used methods include differential centrifugation, density gradient centrifugation, ultrafiltration, polymer precipitation, and immunoaffinity capture (Li et al., 2017; Yang DB et al., 2020). In addition, efforts are still being pursued to improve existing methods and establish new ones. For example, the cushioned-density gradient ultracentrifugation (C-DGUC), a modification of DGUC, could purify exosomes from dense protein aggregates (Li et al., 2018). In addition, size-exclusion chromatography is commonly used to isolate EVs from the serum because it could gain high-purity exosomes (Monguió-Tortajada et al., 2019). Besides, asymmetric-flow field-flow fractionation (AF4) has been applied to isolate EVs and exosomes. Zhang et al. (2018) isolated and characterized two subpopulations of exosomes with AF4, and subsequently named them as large exosome vesicles (90–120 nm) and small exosome vesicles (60–80 nm). However, AF4 requires expertise to customize the protocol (Zhang and Lyden, 2019). Furthermore, other exosomal isolation methods have also shown great potential, such as microfluidic technologies (Hassanpour Tamrin et al., 2021), exosome detection method via the ultrafast-isolation system (EXODUS) (Chen YC et al., 2021), nanostructures and microfluidic devices (Le and Fan, 2021), rapid exosome isolation using ultrafiltration and size exclusion chromatography (REIUS) (la Shu et al., 2021). In short, as each isolation method has its advantages and drawbacks, it is highly practical to choose an appropriate method according to the samples and demands, rather than developing a universal method.

2.2 Exosomal characterization

The heterogeneity of exosomes and the differences between isolation methods, as well as residual non-exosomal substances, present certain requirements for the characterization of isolated exosomes. Traditionally, transmission electron microscopy (TEM), nanoparticle tracer analysis (NTA), and western blotting were used to characterize the isolated exosomes, and additional techniques have been developed for exosomal characterization. Firstly, scanning electron microscopy (SEM) (Gao et al., 2018) and atomic force microscopy (AFM) (Sharma et al., 2018) were adopted for characterizing cup-shaped morphology. With regards to exosome size characterization, single-particle interference reflection imaging sensor (SP-IRIS), microfluidic resistive pulse sensing (MRPS), and nanoflow cytometry measurement (NFCM) are alternative methods to NTA. By comparison among NTA, SP-IRIS, MRPS, and NFCM based on particle size, concentration, and fluorescence measurement accuracy, Arab et al. (2021) suggested that NFCM is the best one that can simultaneously provide particle size distribution, particle concentration, and fluorescence measurement accuracy at the single-particle level. Western blotting is generally applied to characterize exosome-membrane proteins, such as cluster of differentiation 9 (CD9) and CD63. Some researchers suggested excluding the possibility that some impurities mix in exosomes by testing negative biomarkers such as Golgi matrix protein 130 (GM130) and calnexin (Samaeekia et al., 2018). However, given the complexity of exosomal cargos,

there is no commonly recognized biomarker of impurities, and it requires judgement based on samples (Théry et al., 2018). Clearly, it is of great value to develop multiple characterization methods to identify the collected subpopulations of EVs. The precise distinguishment of exosomes from other EVs will favor the development of isolation, function, and the ultimate theranostic therapy of exosome-mediated cell-free strategy.

2.3 Exosomal mechanisms of action

It is well-established that exosomes exhibit regulatory roles through exosomal membrane proteins (Gross et al., 2012; Gao et al., 2016) and cargos targeting the receptors located on the surface (Wang et al., 2011) or inside the recipient (Wei et al., 2020). Recently, Keller et al. (2020) reported a new exosomal mechanism of action: bacterial DNA and cytosine-guanine dinucleotide (CpG) DNA lead to an increased secretion of a disintegrin and metalloproteinase 10 (ADAM10)-bearing exosomes from both human and mice cells, and ADAM10-bearing exosomes act as decoys in response to bacterial infection to prevent damages to target tissues (Fig. 1). This novel mechanism contributes to expanding our understanding of exosomes and exhibits great potential as endogenous decoys in the clinical treatment of virus-induced diseases, especially those of skeletal muscle.

3 Exosome-mediated regulatory mechanisms in skeletal muscle

Accumulating evidence shows that exosomes extensively participate in the physiology and pathology of skeletal muscle. Exosome-mediated cell-free strategy, resembling the beneficial effects of cell-based strategies without immune rejection, has been a popular field to explore the pathobiology and therapeutic strategy in multiple pathophysiologic processes. In this section, we focus our attention on exosomes, but not all EV subsets, derived from multiple tissues and

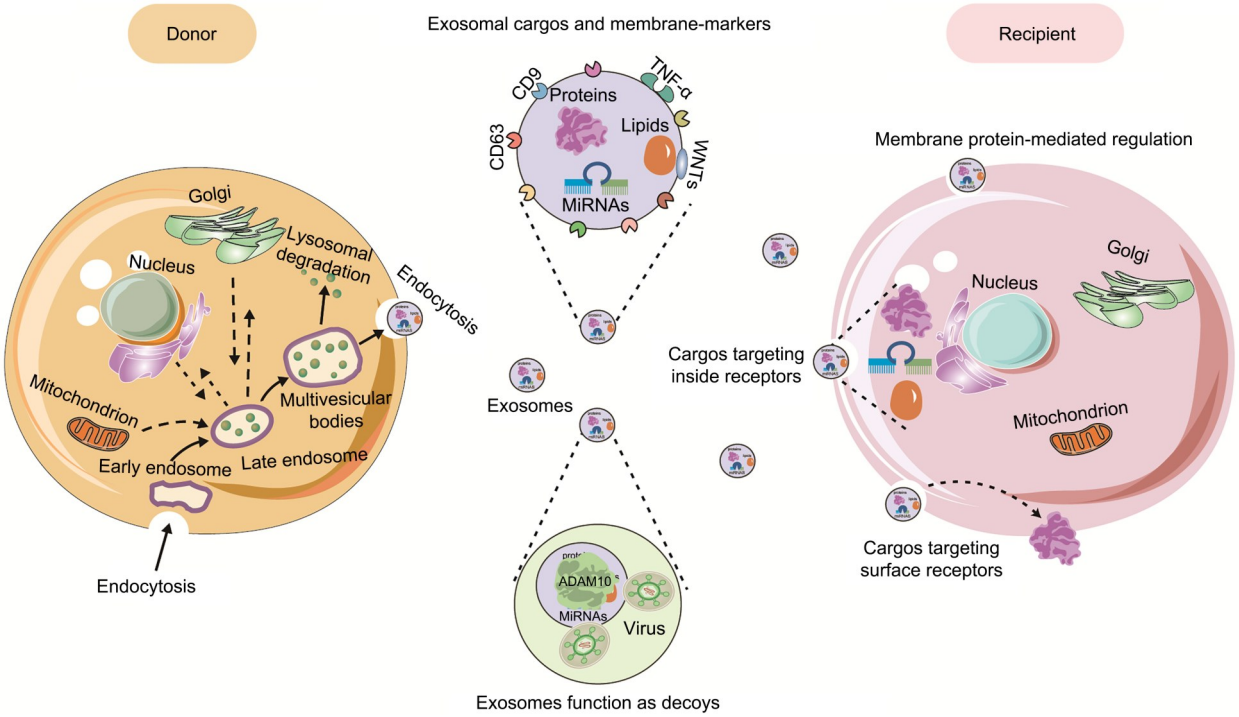

Fig. 1 Exosomal biogenesis and mechanisms of action. Exosomes are described as vesicles of endosomal origin. Endocytosis is the first step to form early endosomes, and the early endosomes mature into late endosomes with specific cargos loaded, which further mature into multivesicular bodies. The latter are subject to two pathways: either lysosomal degradation, or secretion as exosomes with a size of 30–150 nm in diameter through fusion with the plasma membrane and ultimate exocytosis. Cluster of differentiation 9 (CD9) and CD63 are membrane markers; tumor necrosis factor-α (TNF-α) and Wingless/Int-1 lipoglycoproteins (WNTs) are exosomal cargos on the surface. MiRNAs: microRNAs; ADAM10: a disintegrin and metalloproteinase 10.

organs (skeletal muscle, adipose, blood, urine, cancer, mesenchymal stem cells (MSCs), and other types of cells) in regulating the physiological and pathological development of skeletal muscle (Table 1).

Table 1 Biological functions and mechanism of exosomes in skeletal muscle

Origin	Factor	Function	Target	Reference
Myogenic progenitor cells	MiR-206	Inhibits collagen biosynthesis to ensure optimal muscle remodeling	RRBP1	Fry et al., 2017
Hindlimb muscle	Let-7-5p	Increases protein synthesis	IGF-1	Huang et al., 2021
Adipocyte	MiR-27a	Induces IR	PPARγ	Yu et al., 2018; Wang et al., 2022
Gonadal white adipose tissue	MiR-222	Promotes obesity-associated IR and glucose intolerance	IRS1 and p-AKT	Li et al., 2020
Adipose tissue macrophage (M1)	MiR-155	Causes glucose intolerance and IR	PPARγ	Ying et al., 2017
Bone marrow-derived macrophages (M2)	MiR-690	Improves glucose intolerance and insulin sensitivity	*Nadk*	Ying et al., 2021
Adipose tissue macrophage (M1)	MiR-29a	Causes IR	PPARδ	Liu et al., 2019
Serum	MiR-20b-5p	Impairs insulin action in human skeletal muscle	AKTIP and STAT3	Katayama et al., 2019
Oral squamous cell carcinoma cells	MiR-181a-3p	Induces muscle cell atrophy and apoptosis	Unspecified	Qiu et al., 2020b
C26 cell-induced colon cancer	MiR-195a-5p and miR-125b-1-3p	Induce skeletal muscle wasting	Bcl-2	Miao et al., 2021
C26 cells	GDF-15	Induces skeletal muscle atrophy	Bcl-2/ caspase-3	Zhang et al., 2022
Umbilical cord MSCs	Unspecified	Ameliorates blood glucose levels and partially reverses IR	GLUT4	Sun et al., 2018
Placenta-derived MSCs	MiR-29c	Promotes differentiation of myoblasts	Unspecified	Bier et al., 2018
Umbilical cord MSCs	CircHIPK3	Prevents pyroptosis and repair ischemic muscle injury	MiR-421/ FoxO3a	Yan et al., 2020
Bone marrow MSCs	Unspecified	Facilitates recovery after injury	TGF-β	Iyer et al., 2020
Bone marrow MSCs	MiR-486-5p	Inhibits dexamethasone-induced muscle atrophy	FoxO1	Li et al., 2021
Adipose tissue-derived MSCs	Unspecified	Attenuates the damage of biopsy punch to muscle bundles	MYOG/ MYOD	Byun et al., 2021
Muscle-derived fibroblasts	MiR-199a-5p	Promotes skeletal muscle fibrosis	Caveolin-1	Zanotti et al., 2018
Engineered HEK293 cells	MiR-26a	Ameliorates muscle wasting	FoxO1	Zhang et al., 2019
Engineered bone marrow MSCs	MiR-215	Protects skeletal muscle against injury	FABP3	Zhou Q et al., 2021
Engineered C2C12 cells	Hsp60	Ameliorates muscle wasting and cachexia	PGC-1α isoform 1	di Felice et al., 2022
Engineered human bone marrow mesenchymal stromal cells	IL6ST	Partially ameliorates IL6/IL6R complexes-induced anti-differentiation effects	STAT3	Conceição et al., 2021

AKTIP: protein kinase B (AKT)-interacting protein; Bcl-2: B-cell lymphoma-2; CircHIPK3: circular RNA homeodomain-interacting protein kinase 3; FoxO3a: forkhead box O3a; FABP3: fatty acid-binding protein 3 gene; GDF-15: growth differentiation factor-15; GLUT4: glucose transporter 4; Hsp60: heat shock protein 60; IGF-1: insulin-like growth factor-1; IL6R: interleukin 6 (IL6) receptor; IL6ST: IL6 signal transducer; IRS1: insulin receptor substrate 1; IR: insulin resistance; Let-7-5p: lethal-7-5p; MiR: microRNA; MSCs: mesenchymal stem cells; MYOG: myogenin; MYOD: myoblast determination protein 1; *Nadk*: a gene encoding nicotinamide adenine dinucleotide $(NAD)^+$ kinase; p-AKT: phosphorylated protein kinase B; PGC-1α: peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1α; RRBP1: ribosome-binding protein 1; STAT3: signal transducer and activator of transcription 3; TGF-β: transforming growth factor-β.

3.1 Regulatory mechanisms of skeletal muscle-derived exosomes in skeletal muscle

Skeletal muscle secretes exosomes, some of which enter the bloodstream and others are taken up by proximal muscle tissue (Mytidou et al., 2021), to influence its own fate and biological activities, such as muscle remodeling. Fry et al. (2017) illustrated that myogenic progenitor cells (MPCs) contribute to optimal muscle remodeling in response to hypertrophic stimuli via the exosomal microRNA-206 (miR-206)/ribosome-binding protein 1 (RRBP1) axis. Skeletal muscle-derived exosomes are associated with muscle atrophy. Kim et al. (2018) reported that exosomes isolated from the conditioned media (CM) of inflammatory murine myoblast (C2C12) myotubes contribute to inflammation-induced muscle atrophy by causing myoblast inflammation and inhibiting the myogenic process. In addition to inflammatory models, myotube-derived exosomes induce functional improvements and alleviate muscle deterioration by improving muscle membrane integrity in dystrophic mice (Leng et al., 2021), highlighting the therapeutic potential of exosomes for Duchenne muscular dystrophy (DMD) and other skeletal muscle disorders with compromised membranes. Furthermore, Huang et al. (2021) found that acupuncture with low-frequency electrical stimulation (Acu/LFES), a potential strategy to treat muscle atrophy (Hu et al., 2015), promotes protein synthesis in the hindlimb and forelimb skeletal muscles by decreasing the expression of lethal-7-5p (let-7-5p), which inhibits insulin-like growth factor-1 (IGF-1), in the muscle of both hindlimbs and the circulating exosomes of C57/BL6 mice. Moreover, exosomes also exert a pro-fibrotic effect. Zanotti et al. (2018) reported that exosomal miR-199a-5p from muscle-derived fibroblasts in DMD patients targets caveolin-1, an anti-fibrotic signaling molecule (Yi et al., 2014), and induces skeletal muscle fibrosis. Collectively, the above studies indicate that the exosomal cargos, such as miRNAs, are altered in response to physiological and pathological stimuli, and the altered cargos in turn influence the physiological and pathological fates of skeletal muscle, and thus could be applied for the treatment of skeletal muscle diseases.

3.2 Regulatory functions of adipose-derived exosomes in skeletal muscle

Both skeletal muscle and adipose tissue are of mesodermal origin (Buckingham et al., 2003; Billon et al., 2008). Numerous studies, especially those related to exosomes, provided evidence for their close relationships with insulin resistance (IR). Yu et al. (2018) found that adipocyte-derived exosomal miR-27a induces IR in skeletal muscle by inhibiting the expression of peroxisome proliferator-activated receptor γ (PPARγ) and its downstream genes. Besides, Li et al. (2020) found that gonadal white adipose tissue-derived exosomal miR-222 is upregulated in obese patients with IR and in serum samples from type 2 diabetes mellitus (T2DM) patients. The elevated exosomal miR-222 is considered be associated with IR and glucose homeostasis/disposal in skeletal muscle, as it downregulates the insulin receptor substrate 1 (IRS1) and phosphorylated protein kinase B (p-AKT) levels. Macrophages residing within adipose tissue are associated with insulin sensitivity in skeletal muscle. Ying et al. (2017) found that miRNA-containing exosomes from adipose tissue macrophages (ATMs-Exos) of obese mice bring about glucose intolerance and IR in the skeletal muscle of lean mice, whereas ATMs-Exos from lean mice improve the glucose tolerance and insulin sensitivity of obese mice. ATMs-Exo-miR-155 is the key mediator that leads to glucose intolerance and IR in skeletal muscle by targeting PPARγ (Ying et al., 2017), and exosomal miR-690 is the key mediator that improves glucose tolerance and insulin sensitivity in the skeletal muscle of obese mice by targeting *Nadk* (a gene encoding nicotinamide adenine dinucleotide $(NAD)^+$ kinase) (Ying et al., 2021). It is worth noting that Liu et al. (2019) identified another mediator, miR-29a, in ATMs-Exos of obese mice, that causes IR in myocytes by targeting PPARδ. Beyond IR, adipose-derived exosomes play important roles in skeletal muscle regeneration. Wang CY et al. (2019) demonstrated that human adipose stem cells-derived exosomes (ASCs-Exos) significantly improve the regeneration and biomechanical properties of torn rotator cuff muscles by ameliorating atrophy and degeneration. What is more, exosomes from adipose-derived MSCs (AD-MSCs-Exos) promote the proliferation and expression of myocyte-related genes. Importantly, AD-MSCs-Exos attenuate the damage of biopsy punch to the shapes and sizes of the muscle bundles and increase the expression of myogenin (MYOG) and myoblast determination protein 1 (MYOD) (Byun et al., 2021). To sum up, adipose-derived exosome is critical to skeletal muscle IR and regeneration, and

exosomal miRNA is the most widely studied regulator in the current research (Table 1). Given that adipose tissue is composed of multiple cell types, it will be an important study to distinguish the cell types/subtypes that exert beneficial effects through exosomes.

3.3 Regulatory roles of blood-derived exosomes in skeletal muscle

Blood is a circulating body fluid of vertebrates, which contains exosomes (Li et al., 2014; Manterola et al., 2014; Zhao et al., 2017). Experimental studies indicated that serum-EV is a potential factor against muscle damage and aged skeletal muscle atrophy (Cavallari et al., 2017; Sahu et al., 2021). In a rat model with tibialis anterior muscle injury, Iyer et al. (2020) reported that platelet-rich plasma-derived exosomes accelerated the recovery rhythm of contractile function by upregulating MYOG. In addition to muscle regeneration, serum exosome exerts a regulatory effect on T2DM. Katayama et al. (2019) documented that the overexpression of miR-20b-5p, a differentially expressed serum exosomal miRNA between normal subjects and T2DM patients, promotes basal glycogen synthesis in myocytes by targeting AKT-interacting protein (AKTIP) and signal transducer and activator of transcription 3 (STAT3). In older subjects doing long-term exercise, Nair et al. (2020) identified a subset of differentially expressed plasma-derived exosomal miRNAs that target genes in the insulin-like growth factor-1 (IGF-1) signaling pathway, a pathway involved in muscle growth and glucose homeostasis (Vassilakos et al., 2019), highlighting the potential role of exercise-induced circulating exosomes in muscle growth and glucose homeostasis. Furthermore, Wang et al. (2022) reported the exercise-induced reduction of serum exosomal miR-27a level, which is positively associated with IR (Yu et al., 2018) by targeting PPARγ. Briefly, blood exosomes are important mediators of both injury and metabolic disease in skeletal muscle. Considering the wide availability of blood, further studies are warranted to explore the unknown effects of blood exosomes on skeletal muscle, to advance their translational potential in the clinic.

3.4 Regulatory functions of urine-derived exosomes in skeletal muscle

Beyond being a metabolic waste, urine is considered as a promising non-invasive cell source for cell-based therapies in skeletal muscle regeneration (Chen et al., 2017), while exosomal studies have shown that it might be related with cell-free therapies. Zhu et al. (2018) collected urine-derived stem cells-EV (USCs-EV) with a diameter of 30–150 nm (within the range of exosomes) that expressed exosomal markers. The intramuscular injection of USCs-EV promoted muscle regeneration in a hindlimb ischemia mouse model. Furthermore, in vitro trials showed that USCs-EV promotes C2C12 cell proliferation in a dose-dependent manner (Zhu et al., 2018). Using a rat stress urinary incontinence (SUI) model, Wu RY et al. (2019) found that USCs-exosomes (USCs-Exos) significantly accelerate the recovery of pubococcygeus muscle by promoting the activation, proliferation, and differentiation of muscle stem cells. In summary, urinary exosomes play significant roles in skeletal muscle regeneration. Given that urine is probably the most readily available patient-derived substance, it is likely to become a vital cell-source for exosome-mediated clinical treatments for skeletal muscle diseases.

3.5 Regulatory mechanisms of cancer-derived exosomes in skeletal muscle

Cancer cachexia is a cancer-induced systemic multifactorial disease characterized by specific losses of skeletal muscle, muscle strength, and adipose tissue that deteriorates the quality of life and increases the mortality of patients (Baracos et al., 2018; Schmidt et al., 2018). Recent studies on exosomes may accelerate the development of therapeutic interventions to prevent or reverse this muscle wasting syndrome (Li et al., 2016; Hu et al., 2019). EVs play a role in endoplasmic reticulum stress (ERS)-induced deterioration by promoting the malignant transformation of predisposed cells (Wu CH et al., 2019) and cancer cell apoptosis (Qiu et al., 2020a). Qiu et al. (2020b) further reported that exosomes from the CM of oral squamous cell carcinoma cells treated by tunicamycin lead to muscle atrophy and apoptosis by mediating transmissible ERS signaling. Exosomal miR-181a-3p was subsequently identified as a key factor. Circulating exosome intensity is relevant to cancer cachexia. Miao et al. (2021) showed that C26 colon carcinoma cell-derived exosomal miR-195a-5p and miR-125b-1-3p decrease the sizes of C2C12 myotubes and diminish muscle strength and muscle weight in C26 tumor-bearing mice by reducing the expression of B-cell lymphoma-2 (Bcl-2), an anti-apoptosis protein (Schöneich et al.,

2014), at both the messenger RNA (mRNA) and protein levels. In a subsequent study, Zhang et al. (2022) elucidated that growth differentiation factor-15 (GDF-15) is enriched in C26 colon carcinoma cell-derived exosomes (C26-Exos). The overexpression of GDF-15 in non-cachexic MC38-derived exosomes increases the potency of inducing muscle atrophy, whereas the knockdown of GDF-15 weakens the ability of C26-Exos to trigger muscle atrophy. Mechanistically, GDF-15 contributes to muscle atrophy via targeting Bcl-2/caspase-3 pathways (Zhang et al., 2022). Zhou L et al. (2021) generated colon cancer cachexia and lung cancer cachexia mice models by the subcutaneous injection of CT26 and Lewis lung carcinoma (LLC) tumor cells, respectively. Importantly, both models showed increased circulating levels of exosomes that were largely derived from tumors. Meanwhile, amiloride, a potassium-sparing diuretic with anticancer effect (Rojas et al., 2017), markedly alleviated muscle atrophy, and inhibited the secretion of tumor-derived exosomes without inducing systemic toxicities in healthy mice, highlighting the role of tumor-derived exosome in cancer cachexia (Zhou L et al., 2021). In short, these studies suggest that cancer-derived exosomal cargos (such as miRNAs and proteins) and their circulating levels change with pathological development, providing a significant clinical value for muscle atrophy in cancer cachexia by inhibiting the production of tumor exosomes.

3.6 Regulatory roles of mesenchymal stem cell-derived exosomes in skeletal muscle

MSCs are multipotent—that is, they possess great potential to differentiate into multiple types of cells. Because MSCs are easily obtained and cultured in vitro, there is a remarkable desire to test whether they exert beneficial effects on specific diseases (Wu et al., 2018; Fu et al., 2019). Sun et al. (2018) reported that the exosomes of human umbilical cord-derived MSC (hucMSC-Exos) ameliorate blood glucose levels and partially reverse IR in T2DM, that is, at least in part, to be mediated by upregulating the expression of membrane translocation of glucose transporter 4 (GLUT4) in muscle. Apart from IR, MSCs-derived exosomes play a role in muscle injury. Bier et al. (2018) found that CM and exosomes isolated from placenta-derived MSCs (PL-MSCs) promote the differentiation of myoblasts from Duchenne patients and mdx mice, and decrease the expression of fibrogenic genes in myoblasts of patients with DMD; the miR-29c at least partially mediates the effects of CM and exosome. Furthermore, Yan et al. (2020) showed that hucMSC-Exo treatment significantly promotes running distance and muscle force in a hindlimb ischemia mice model, which are blocked by the injection of human umbilical cord MSC-derived exosomes (UMSC-Exos) and small interfering RNA (siRNA)-targeting circular RNA homeodomain-interacting protein kinase 3 (si-circHIPK3). Besides, Iyer et al. (2020) revealed that muscles treated with exosomes of bone marrow-derived MSC (BMSC-Exos) reduce the expression of transforming growth factor-β (TGF-β) in a muscle injury rat model, while Li et al. (2021) reported that BMSC-Exos blunt dexamethasone-induced muscle atrophy both in vitro and in vivo via the miR-486-5p/forkhead box O1 (FoxO1) axis. In conclusion, the above studies indicate that MSCs-derived exosomes exert beneficial roles against IR and skeletal muscle injury; therefore, it is valuable to search for other types of stem cells that exert beneficial effects on skeletal muscle diseases through exosomes.

3.7 Regulatory activity of exosomes derived from other tissues and cells in skeletal muscle

Exosomes are secreted by almost all living cells and can be detected in various body fluids. Tavakoli Dargani et al. (2018) showed that embryonic stem cell exosomes (ES-Exos) inhibit doxorubicin- and inflammation-induced pyroptosis in mouse soleus muscle cells (Sol 8), while the exosome inhibitor GW4869 abolishes the beneficial effect of ES-Exos.

Circulating placental exosomes are increased in pregnancies with gestational diabetes mellitus (GDM), which are associated with skeletal muscle IR. Nair et al. (2018) reported that GDM pregnancy-derived placental exosomes attenuate the migration and glucose uptake of primary skeletal muscle from normal glucose tolerant (NGT) subjects in response to insulin stimulation, whereas placental exosomes from NGT enhance the migration and glucose uptake of skeletal muscle from diabetic patients.

Furthermore, Aminzadeh et al. (2018) reported that the absence of dystrophin deteriorates exercise ability and increases mortality in DMD patients, while cardiosphere-derived cells (CDCs) and CDC-derived exosomes (CDC-Exos) improve both cardiac and skeletal muscle structure and exercise ability in DMD

patients. In particular, CDC-Exos markedly promote myofiber proliferation, myoblast determination and differentiation three weeks after intrasoleus injection, indicative of enhanced muscle regeneration. Meanwhile, the intrasoleus injection of CDC-Exos decreases inflammation and fibrosis, highlighting the therapeutic potential of CDC-Exos for DMD patients (Aminzadeh et al., 2018).

In brief, a massive number of cell types communicate with skeletal muscle via exosomes, so more attention should be paid to exploring additional types of cells that communicate with skeletal muscle via exosomes.

4 Engineered exosomes in skeletal muscle

Exosomes are widely involved in the physiology and pathology of skeletal muscle and hold great clinical potential in the treatment of skeletal muscle diseases. However, a large number of donors are necessary to induce sufficient effects in vivo due to the low purity and insufficient yield of exosomes (Ying et al., 2017, 2021). Artificially engineered exosomes possess tissue-specific targeting capability and modified cargos, and thus may be a donor-saving approach for in vivo studies. MiR-26a is required for skeletal muscle differentiation and regeneration (Dey et al., 2012). Zhang et al. (2019) showed that the intramuscular injection of miR-26a-containing exosomes generated by transfecting the Lamp2b-miR-26a vector into HEK293 cells, ameliorates the muscle wasting of unilateral ureteral obstruction mice by targeting FoxO1. Wang B et al. (2019) generated miR-26a-overexpressing exosomes (Exo/miR-26a) containing a muscle-specific targeting peptide that helps them to be selectively delivered to muscle cells. The intramuscular injection of Exo/miR-26a prevents chronic kidney disease (CKD)-induced muscle wasting in a 5/6 nephrectomized CKD mice model. MiR-215, a tumor inhibitor (Ge et al., 2016), exhibits protective effects against cancer cachexia-induced muscle atrophy. Zhou Q et al. (2021) developed exosomes overexpressing miR-215 (miR-215-modified exosomes) by transfecting miR-215 mimics into rat bone marrow MSCs (rBMSCs), and the miR-215-modified exosomes reversed the H_2O_2-induced inhibition of cell viability and increased the apoptosis of L6 cells. In addition to miRNAs, exosomal proteins also exert bioactive effects. Heat shock protein 60 (Hsp60)-bearing exosomes are reported to induce PPARγ coactivator-α1 (PGC-1α) expression (Barone et al., 2016). di Felice et al. (2022) generated Hsp60-bearing exosomes (with a diameter of 50–140 nm) by genetically modifying C2C12 cell lines with an Hsp60-overexpressing plasmid. The obtained exosomes activated the expression of PGC-1α isoform 1, which is directly involved in mitochondrial biogenesis and muscle atrophy suppression, in C2C12 cell lines. In addition to delivering biomolecules, engineered exosomes could regulate skeletal muscle development as a decoy receptor. Conceição et al. (2021) generated engineered EVs (average diameter of 144 nm, also known as exosomes) to express interleukin 6 (IL6) signal transducer (IL6ST) decoy receptors that selectively inhibit the IL6 trans-signaling pathway in C2C12 myoblasts and myotubes, thereby partially ameliorating the IL6/IL6 receptor (IL6R) complexes-induced anti-differentiation effects. On the whole, the engineered exosomes with modified contents and tissue-specific targeting capability present a tremendous application prospect in the treatment of muscle diseases.

5 Conclusions

Exosomes comprise the most widely investigated subset among the four main subsets of EVs (exosomes, microvesicles, apoptotic body, and oncosome), and their functions are beyond the initial expectations. However, in addition to their poor yield and insufficient purity, there are still a number of concerns regarding exosomes. Firstly, several tissues/organs deserve attention regarding the exosome-mediated regulation of skeletal muscle. For example, the nervous system plays an important role in skeletal muscle development (Yang XF et al., 2020), while little is known about the exosome-mediated regulatory mechanisms of the nervous system in skeletal muscle. Secondly, gut microbes are critical for skeletal muscle. Choi et al. (2015) reported that stool EVs from high-fat diet (HFD)-fed mice impair glucose metabolism in skeletal muscle, which revealed a subject of intense study by elaborating the unknown effects of gut microbe-derived exosomes on skeletal muscle. Thirdly, the source of exosomes is limited, which indirectly burdens the investigation and clinical application of exosomes. Since many organisms, including archaea (Evguenieva-Hackenberg et al., 2014), bacteria (Deng et al., 2015), fungi (Oliveira

et al., 2013), and plant cells (Xu et al., 2021), secrete exosomes, the comparison results between different sources of exosomes from cells and animals to milk, bacteria, fungi, and plants warrant further investigations. At last, due to its relatively low immune rejection rate compared with cell-based therapy, trials for exosome-based translational medicine could become a hot topic, while the clinical application of engineered exosomes is undeveloped and insufficient. Native exosomes contain a specific combination of proteins, nucleic acids, and lipids, while the engineered exosomes are typically modified for a single bio-molecule, which is considered insufficient and could be a time-wasting operation compared with mimics and recombinant proteins for clinical treatment. The combination of proteins, nucleic acids, lipids, and drugs may be the future of clinical applications. Notably, decoy receptors may be another application strategy.

As comprehensively discussed in this work, advances in the research on exosome-mediated cellular bio-information exchange and reprogramming of skeletal muscle cells have expanded our knowledge. On the one hand, the cargos and circulating levels of exosomes change in response to physiological and pathological stimuli, and these changes in turn affect the physiological and pathological fates of skeletal muscle. On the other hand, engineered exosomes with modified contents and tissue-specific targeting ligands hold tremendous promise for targeted therapy in treating skeletal muscle diseases (Fig. 2). We believe that those marked advances provide extraordinary opportunities to deepen the understanding and boost the clinical applications of exosomes to tackle skeletal muscle diseases.

Review criteria

We searched PubMed for original, full-length, English-language articles published within the last five years (2017–2022). The search terms (individually or in combination) included: “extracellular vesicle,” “exosome,” “exosome isolation,” “muscle,” “skeletal muscle,” “myotube,” “C2C12,” “myogenesis,” “muscle atrophy,” and “muscle loss.” We preferred those original articles that report novel discovery in the exosome field and the exosomal function and/or mechanism relevant to skeletal muscle. The professional opinions of each of the authors were considered in the selection of articles relevant to this review, and all listed references were evaluated by at least one of the authors.

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Author contributions

Gongshe YANG and Xin’e SHI outlined the content of the review and made the final corrections for the manuscript. Zhaolu WANG, Jinjin YANG, Xiaohui SUN, and Xi SUN collected the literature and wrote the manuscript. Zhaolu

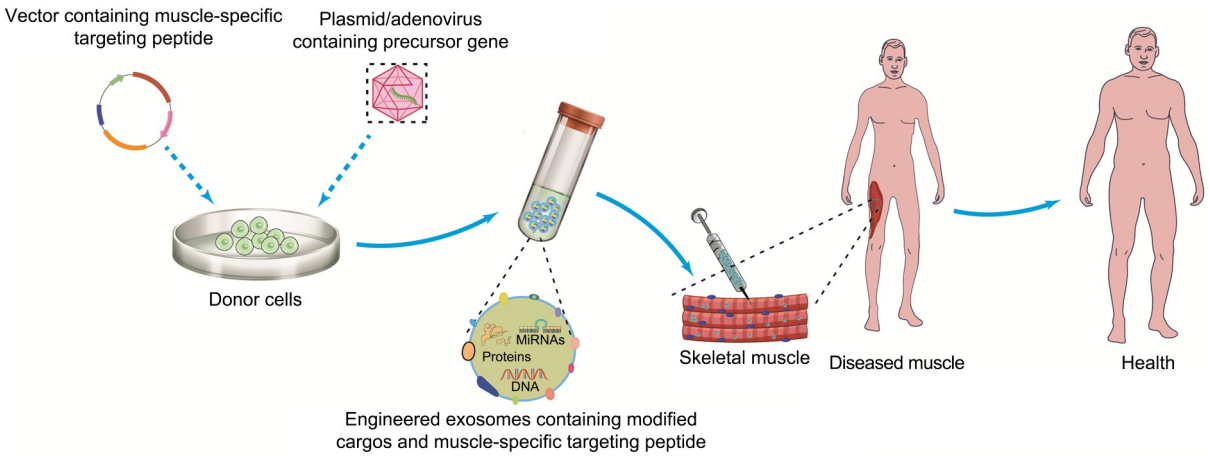

Fig. 2 Graphical abstract of potential clinical applications of engineered exosomes. A vector containing a muscle-specific targeting peptide and plasmid/adenovirus containing a precursor gene are co-transfected into donor cells, and then the donor cells are anticipated to secrete engineered exosomes with muscle-specific targeting capability and combined cargos such as proteins, nucleic acids, lipids, and drugs. These engineered exosomes can provide targeted therapy for the treatment of skeletal muscle diseases.

WANG drew the figures. All authors have read and agreed the final manuscript.

Compliance with ethics guidelines

Zhaolu WANG, Jinjin YANG, Xiaohui SUN, Xi SUN, Gongshe YANG, and Xin'e SHI declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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