Skeletal muscle-derived extracellular vesicles in multi-organ degenerative disease: Mechanisms and therapeutic delivery perspectives (Review)

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Abstract. Multi-organ degenerative diseases are age-associated or chronic disorders marked by progressive tissue deterioration, impaired repair and functional decline, with representative conditions including sarcopenia, osteoporosis, osteoarthritis, neurodegenerative or ischemia-associated neurological disorders, heart failure, chronic kidney disease and diabetes-associated tissue dysfunction. Their frequent coexistence in aging populations limits the effectiveness of therapeutic strategies directed at a single organ or pathway. Extracellular vesicles (EVs) are lipid bilayer-enclosed particles that shuttle proteins, lipids, metabolites and regulatory RNAs between cells and tissue. As a highly metabolic and secretory tissue, skeletal muscle releases skeletal muscle-derived EVs (SkM-EVs) that may carry muscle-enriched microRNAs, together with other regulatory cargo molecules involved in local tissue remodeling and systemic signaling. SkM-EVs have therefore been proposed as mediators of muscle-centered cross-organ communication and potential delivery vehicles for molecular intervention, although therapeutic evidence remains largely preclinical. The present review examines the biological functions of SkM-EVs, their regulation by exercise, aging and metabolic stress and their potential involvement in multi-organ degenerative diseases. The present study aimed to discuss engineering strategies for SkM-EVs, including cargo loading, surface modification and targeted delivery, with particular attention to controversies, methodological limitations, quality control requirements and barriers to clinical translation.

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Contents

1. Introduction

Physiological homeostasis depends on coordinated communication between cells, tissue and organ systems and integrates metabolic adaptation, tissue repair and responses to pathological stress. Extracellular vesicles (EVs), lipid bilayer-enclosed particles released by cells into the extracellular space, protect and transfer proteins, lipids, metabolites and nucleic acids between cells, thereby contributing to long-range molecular communication (1,2). According to the Minimal Information for Studies of Extracellular Vesicles 2023 (MISEV2023) guidelines, EVs should be described using operational terms

based on physical characteristics, biochemical composition or cell of origin unless their biogenesis has been directly demonstrated (3). Endosomes are intracellular organelles involved in exosome biogenesis and are not regarded as EV subtypes. Skeletal muscle-derived EVs (SkM-EVs) is an umbrella term.

SkM is one of the largest metabolic and secretory tissues in the body and communicates with local and distant organs through endocrine, paracrine and autocrine mechanisms. SkM-EVs contain bioactive cargo molecules, including proteins, peptides, nucleic acids, metabolites and muscle-enriched microRNAs (myomiRs) and may act locally or enter the circulation under specific physiological or pathological conditions (4). This vesicle-mediated mode of signaling extends the classical myokine paradigm by incorporating protected cargo transfer, vesicle heterogeneity and potential tissue-selective delivery (5). Elucidating how SkM-EVs influence distant organs may refine understanding of integrative physiology beyond classical neural, hormonal and soluble myokine signaling. Accordingly, SkM-EVs are increasingly viewed as candidate mediators of muscle-centered interorgan communication and potential delivery vehicles for molecular intervention (4,6,7), although their therapeutic application remains largely preclinical.

In the present review, multi-organ degenerative diseases are operationally defined as age-associated or chronic pathological conditions characterized by progressive structural remodeling, impaired tissue repair and functional decline across one or more organ systems (8). Representative conditions include sarcopenia, osteoporosis, osteoarthritis, neurodegenerative or ischemia-associated neural disorder, heart failure, chronic kidney disease (CKD) and diabetes-associated tissue dysfunction (9-14). These diseases typically share convergent mechanisms, including mitochondrial dysfunction, chronic low-grade inflammation, cell senescence, metabolic dysregulation, impaired proteostasis and altered intercellular communication (15). Given the rapid expansion of EV biology and persistent challenges in EV nomenclature, source attribution, isolation, characterization, biodistribution and clinical translation (3,16), the present review aimed to summarize the biological functions of SkM-EVs, their context-dependent regulation in response to exercise, aging and metabolic stress and their potential roles as mediators and delivery platforms in multi-organ degenerative diseases, as well as controversies, methodological limitations and translational barriers to provide a framework for future SkM-EV research.

2. Biogenesis, structural features and functional cargo of SkM-EVs

Biogenesis and structural features of SkM-EVs. EVs comprise heterogeneous vesicle populations that differ in size, biogenesis, molecular cargo and release mechanisms. Biogenesis-defined exosomes originate as intraluminal vesicles formed during multivesicular body maturation and are released following fusion with the plasma membrane, whereas microvesicles, also termed ectosomes, arise from outward budding and fission of the plasma membrane (17,18). Accordingly, the term SkM-EVs is used hereafter as a term for EVs released by SkM cells, differentiated myotubes or SkM tissue, rather than as a biogenesis-defined EV subtype.

SkM constitutes ~40% of body mass in healthy adults and represents a major metabolic and secretory tissue (19). Guescini *et al* (20) showed that SkM cells can release EVs containing mitochondrial DNA and proteins involved in signal transduction. Similarly to EVs from other cell types, SkM-EVs contain lipid bilayers and carry diverse cargo molecules, including membrane proteins, receptors, enzymes, extracellular matrix-related proteins and nucleic acids such as DNA, mRNA and miRs (21,22). These cargo molecules enable SkM-EVs to transfer molecular signals to neighboring or distant recipient cells and may contribute to extracellular matrix remodeling, metabolic regulation and tissue repair (23).

Compared with EVs derived from non-muscle tissue, SkM-EVs may contain muscle-associated cargo molecules that reflect the physiological or pathological state of SkM. These include cytoskeletal and contractile proteins, such as actin, troponin and myosin-associated proteins, as well as myogenic differentiation-associated proteins or transcripts (24). However, these molecules should be interpreted as muscle-associated rather than SkM-specific markers, particularly in circulating EV preparations where vesicles from multiple tissues coexist. Therefore, muscle-enriched cargo molecules should be used together with EV-associated markers and source-validation approaches to support the involvement of SkM-EVs in muscle growth, repair and regeneration.

Isolation, enrichment and characterization methods for SkM-EVs. SkM-EVs are mainly enriched from SkM cell-conditioned medium, primary myotube cultures and SkM interstitial fluid (7,25). Serum or plasma EV preparations are sometimes used to infer muscle-associated EV signatures, but circulating EVs contain vesicles from multiple tissue types and therefore cannot be regarded as SkM-EV preparations without additional source validation (26). Separation strategies, including differential ultracentrifugation, density-gradient centrifugation, ultrafiltration, size-exclusion chromatography, polymer-based precipitation, immunoaffinity capture and microfluidic approaches, differ in vesicle yield, apparent purity, cargo recovery and scalability (27). These methodological differences are not merely technical details, because co-isolated soluble proteins, lipoproteins, ribonucleoprotein complexes or other non-vesicular particles may influence downstream omics profiling and functional assays (28). Therefore, the choice of isolation or enrichment strategy should be aligned with the biological question, sample source, expected EV abundance, downstream cargo analysis and functional validation design.

Reliable characterization of SkM-EVs requires integrated physical, biochemical and functional evidence rather than reliance on a single marker or technique. Particle analysis, electron microscopy and EV-enriched markers including CD9, CD63, CD81, tumor susceptibility gene 101 protein (TSG101), ALG-2-interacting protein X (ALIX) and syntenin, support the presence of EVs, whereas negative or contaminant markers should be selected according to sample type to assess preparation quality (3,28). Muscle-associated molecules, including α-sarcoglycan, actin, myosin- and myogenic differentiation-associated proteins or transcripts and myomiRs, may support SkM relevance, but they are not definitive

source markers when used alone (7,25,26). This limitation is important for circulating EV studies because circulating EV preparations contain heterogeneous vesicle populations derived from multiple tissue sources (3,29). Stronger source attribution requires muscle-specific labeling, genetic tracing, tissue enrichment strategies or other orthogonal validation approaches. Functional interpretation should be supported by uptake assays, recipient-cell responses and, where possible, cargo depletion or rescue experiments to establish causal activity.

MyomiRs in SkM-EV-mediated cross-organ signaling: Tissue specificity and source attribution. MyomiRs are muscle-enriched regulatory miRs that participate in myogenesis, muscle remodeling and muscle-associated intercellular communication (30). The best-characterized myomiRs in SkM-EV biology include miR-1, miR-133a, miR-133b and miR-206 (31). However, their tissue specificity is not identical. miR-1 and miR-133a are enriched in striated muscle and are expressed in both skeletal and cardiac muscle, whereas miR-206 shows a SkM-biased expression pattern and is commonly regarded as a SkM-preferential myomiR (32). miR-133b is associated with SkM differentiation and is functionally associated with miR-206 in myogenic differentiation and SkM remodeling, although it should still be interpreted as a SkM-associated rather than strictly SkM-specific miR (33,34). Therefore, these myomiRs should be described as muscle-enriched or -associated cargo molecules rather than uniformly SkM-specific. This distinction is important in cross-organ EV signaling because these myomiRs are typically used to infer muscle-derived vesicular signals in circulating or recipient-tissue EV analyses, whereas their incomplete tissue specificity may confound source attribution (35). Detection of miR-1, miR-133a, miR-133b or miR-206 in circulating EVs may therefore support the involvement of muscle-associated signals, especially in muscle injury, exercise adaptation or muscle remodeling (31,36). Nevertheless, circulating myomiRs alone cannot definitively establish SkM origin because certain myomiRs are shared by skeletal and cardiac muscle and circulating EV pools may contain vesicles from multiple tissues (37,38). Stronger source attribution requires additional evidence, such as SkM-specific labeling, genetic tracing, muscle-associated EV markers, tissue-specific EV enrichment or muscle-directed perturbation of EV release or cargo loading (26). Thus, myomiRs are best interpreted as muscle-enriched cargo molecules that may contribute to SkM-EV-mediated cross-organ communication, whereas their use as source markers requires validation.

Cargo composition and biological functions of SkM-EVs. SkM communicates with local and distant tissue through both soluble myokines and EV-associated cargo molecules. SkM-EVs provide a vesicular route for this communication by carrying bioactive proteins, RNAs, lipids and metabolites that influence recipient cell function in an autocrine, paracrine or endocrine manner (39,40). During myoblast proliferation and differentiation, SkM cells release proteins and peptides as part of the broader secretome, which may act in parallel or interact with EV-mediated signaling (41). Thus, SkM-EVs extend the classical myokine paradigm by enabling protected cargo transfer, vesicle heterogeneity and potential tissue-selective delivery.

The biological activity of SkM-EVs is shaped by cargo composition, surface molecules, donor cell state and recipient cell context. Proteomic analyses have identified EV-associated and surface-enriched proteins, including integrins, heat shock proteins such as HSP60, HSP70 and HSP90 and tetraspanins such as CD9, CD63 and CD81 (42-44). These proteins are involved in vesicle trafficking, membrane organization, cell adhesion, stress responses and intercellular communication (40). In SkM-EVs, muscle-associated proteins and regulatory factors may reflect the physiological or pathological state of the donor muscle, linking vesicle cargo profiles with muscle remodeling, repair and metabolic adaptation (35,45,46).

SkM-EVs may intersect with soluble myokine signaling. Soluble myokines and metabolites, including β-aminoisobutyric acid, fibroblast growth factor 21 (FGF21), vascular endothelial growth factor, brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1), myostatin (MSTN), interleukin-6 (IL-6), -7 and -15 and irisin, have been implicated in muscle-centered interorgan communication (47-49). However, these factors should not be interpreted as definitive SkM-EV cargo molecules unless vesicular association has been experimentally demonstrated. For example, Guo *et al* (50) reported that peptide myokines and EV-associated miRs participate in muscle-adipose communication and systemic metabolism, whereas Hamrick *et al* (51) showed that muscle-derived IGF-1 interacts with IGF-1 receptor (R)-expressing periosteal bone cells to promote osteogenesis during repair. These findings underscore the need to distinguish soluble mediators from vesicle-associated cargo molecules.

RNA cargo molecules represent a major functional component of SkM-EVs. SkM-EVs contain miRs and other small RNAs involved in calcium signaling, neuromuscular communication, SkM mass regulation and tissue remodeling (52). Mytidou *et al* (6) isolated EVs from intact SkM tissue and demonstrated they encapsulate canonical myomiRs, including miR-1, miR-133a, miR-133b and miR-206, supporting EV-mediated local communication within SkM. As aforementioned, these myomiRs should be interpreted as muscle-enriched regulatory cargo molecules rather than uniformly SkM-specific markers, particularly in circulating EV studies (Fig. 1).

The lipid composition of SkM-EVs may contribute to vesicle stability, membrane organization and recipient-cell interactions. Reported lipid components include sphingolipids, cholesterol and oleic, lauric, palmitic, palmitoleic and stearic acid (53). However, compared with protein and RNA cargo molecules, the functional contribution of SkM-EV lipids remains less well defined and requires further lipidomic and mechanistic validation. Together, protein, RNA and lipid cargo molecules provide the molecular basis for SkM-EV-mediated local muscle remodeling and cross-organ communication.

3. SkM-EVs in local muscle remodeling and musculoskeletal crosstalk

SkM-EVs are not restricted to local muscle signaling but may participate in a broader musculoskeletal communication network (54). Within SkM, they modulate myoblast

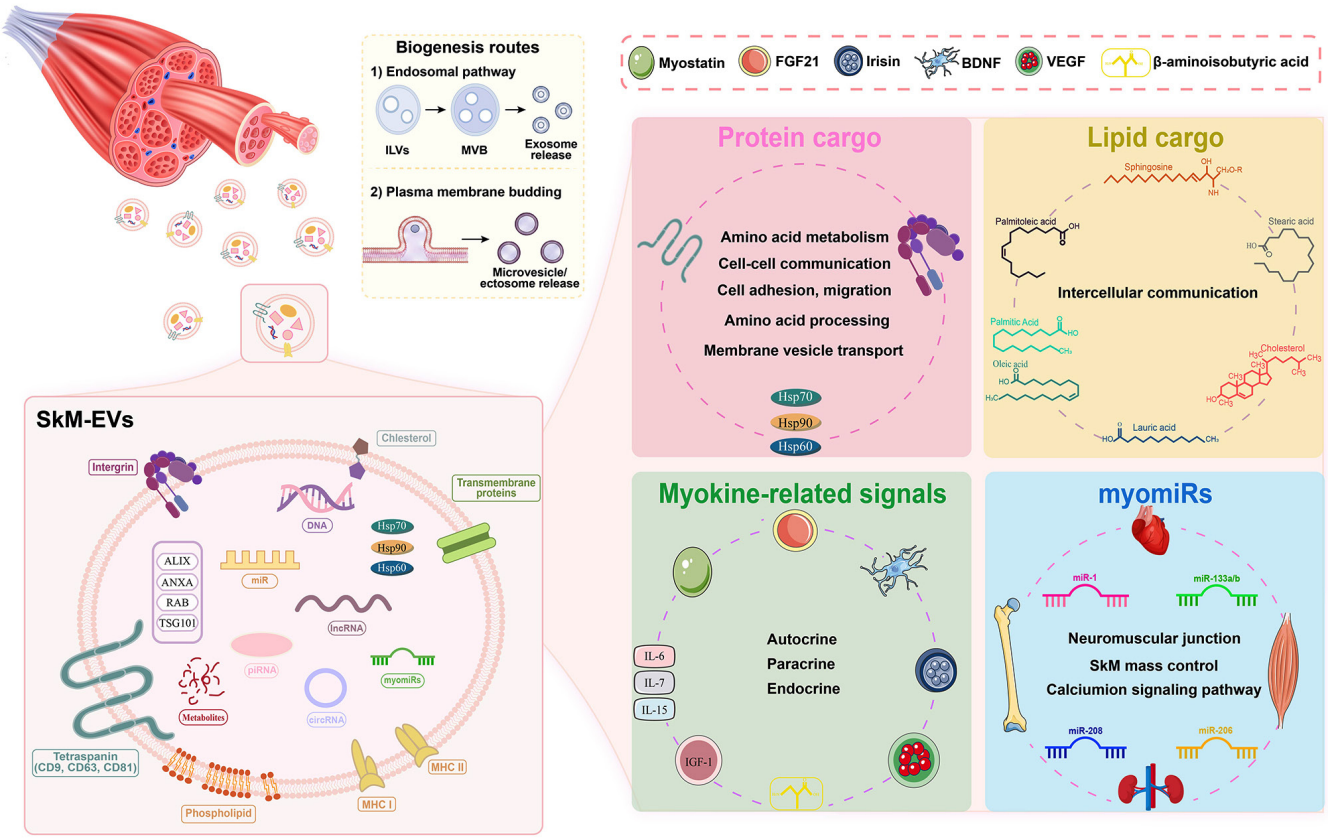

Figure 1. Biogenesis, cargo composition and biological functions of SkM-EVs. SkM-EVs are released through endosomal multivesicular body-associated exosome release or plasma membrane budding-associated microvesicle formation. SkM-EVs carry EV- and muscle-associated proteins, DNA, mRNAs, circRNAs, miRs, lipids and metabolites, including tetraspanins, integrins, HSPs, myomiRs, sphingolipids, cholesterol and fatty acids. These cargo molecules are associated with vesicle transport, membrane organization, recipient cell interactions, SkM remodeling, metabolic adaptation and interorgan communication. SkM-EV, skeletal muscle-derived extracellular vesicle; myomiR, muscle-enriched microRNA; circRNA, circular RNA; ILV, intraluminal vesicle; MVB, multivesicular body; ALIX, ALG-2-interacting protein X; ANXA1, annexin A1; TSG101, tumor susceptibility gene 101 protein; MHC, myosin heavy chain; lncRNA, long non-coding RNA; HSP, heat shock protein; FGF, fibroblast growth factor; BDNF, brain-derived neurotrophic factor.

proliferation, myogenic differentiation, satellite cell activation, extracellular matrix remodeling and atrophy-associated signaling through autocrine and paracrine mechanisms (55). Cargo molecules involved in local muscle remodeling, including myomiRs, transcriptional regulators, metabolic enzymes and other vesicle-associated regulatory factors, may also influence bone marrow mesenchymal stem cells (BMSCs), osteoblasts, osteoclasts and chondrocytes (56,57). Thus, intramuscular EV signaling and muscle-bone crosstalk should be viewed as functionally connected components of musculoskeletal homeostasis. Representative SkM-EV-associated signals, target processes and functional outcomes involved in SkM remodeling and musculoskeletal crosstalk are summarized in Table I.

Autocrine and paracrine roles of SkM-EVs in SkM remodeling. SkM-EVs participate in SkM remodeling primarily through local autocrine and paracrine communication between myofibers, myoblasts, muscle satellite cells, fibroblasts and immune cells (6,58). During myogenic differentiation, myotube-derived EVs deliver proteins and regulatory miRs to myoblasts, thereby influencing cell cycle progression, myogenic commitment and myotube maturation (43,59). In C2C12 cells, Forterre *et al* (43,44) identified muscle-enriched proteins in myoblast- and myotube-derived EVs originally described as exosome-like vesicles and showed that myotube-derived vesicles decreased cyclin D1 expression while increasing myogenin expression, suggesting a role in the transition from myoblast proliferation to differentiation. Myogenic progenitor cell-derived EVs carrying miR-140-5p activate quiescent muscle satellite cells and promote SkM regeneration following injury (55). These findings support a role for SkM-EVs in coordinating progenitor cell activation, myogenic differentiation and early regenerative remodeling.

SkM-EVs contribute to extracellular matrix remodeling and fibrosis-associated responses during muscle repair. Fry *et al* (58) reported that myogenic progenitor cells secrete miR-206-enriched EVs that regulate fibroblast collagen expression and support extracellular matrix remodeling during SkM hypertrophy. Satellite cell-derived EVs are implicated in the regulation of TGF-β1/Smad3-related remodeling responses in denervation-induced muscle atrophy and fibrosis, while TGF-β1/Smad3 signaling has also been linked to muscle wasting, fibrotic remodeling and impaired satellite cell responses in other experimental models (60-62). In the mdx mouse model of dystrophic muscle injury, myotube-derived EV administration improves membrane integrity and muscle function, indicating a potential protective role in dystrophic muscle injury (63). These preclinical findings suggest that SkM-EVs

Table I. Representative SkM-EV signals in skeletal muscle remodeling and musculoskeletal crosstalk.

Source	Key signal	Target/process	Primary outcomes	Evidence level	(Refs.)
C2C12 myoblast/myotube derived EVs	Proteins	Myoblasts	Cyclin D1 decreased, myogenin increased	Cell	(43,59)
MPC-derived EVs	miR-140-5p	Satellite cells	Activation and regeneration enhanced	Animal	(55)
C2C12 EVs	Prrx2	BMSCs	MIR22HG/YAP1 activation, osteogenesis enhanced	Animal	(56)
SkM-EVs	LDHA	BMSC glycolysis	Osteogenic markers increased	Animal	(57)
MPC-derived EVs	miR-206	Fibroblasts	Collagen remodeling, fibrosis restrained	Animal	(58)
Myotube-derived EVs	EXOmyo cargo	Dystrophic muscle	Membrane integrity and muscle function improved	Animal	(63)
EV-mediated delivery	miR-26a	Tibialis anterior muscle	Atrophy decreased, wasting attenuated	Animal	(64)
Inflammatory myotube EVs	Disease-conditioned cargo	Myogenesis/atrophy pathways	MyoD/myogenin levels decreased, stress signaling increased	Cell	(66)
Glucocorticoid atrophy	Extracellular miR-23a	Calcineurin/NFAT, atrophy-associated genes	Calcineurin/NFAT decreased, atrogene expression increased	Cell	(68)
Lipotoxic or insulin-resistant context	Altered EV cargo	Myoblast homeostasis	Homeostasis impaired	Cell	(71)
C2C12 myoblast EVs	miR-27a-3p	MC3T3-E1 cells	SFRP1 decreased, Wnt/β-catenin activation	Cell	(81)
Irisin-enriched EVs	FNDC5/irisin cargo	Osteoblasts	Ferroptosis decreased, proliferation enhanced	Animal	(82)
Myo-EVs	Regulatory cargo	Osteoclasts	TRAP/CTSK decreased, NFATc1 translocation reduced	Cell	(84)

BMSC, bone marrow mesenchymal stem cell; CTSK, cathepsin K; DMD, Duchenne muscular dystrophy; FNDC5, fibronectin type III domain-containing protein 5; LDHA, lactate dehydrogenase A; miR, microRNA; MPC, myogenic progenitor cell; NFAT, nuclear factor of activated T cells; Prrx2, paired related homeobox 2; SkM-EV, skeletal muscle-derived extracellular vesicle; SFRP1, secreted frizzled-related protein 1; TRAP, tartrate-resistant acid phosphatase; EXOmyo, myotube-derived exosomes; MYOD1, myogenic differentiation 1; MIR22HG, MIR22 host gene.

support muscle repair by modulating myogenic differentiation, extracellular matrix organization and membrane stability.

EV-based delivery strategies demonstrate the functional relevance of EV-associated cargo molecules in SkM repair and wasting. In an experimental CKD model, EV-mediated miR-26a delivery to tibialis anterior muscle attenuates muscle wasting, improves insulin resistance-related signaling and decreases the expression of atrophy-associated genes, including F-box protein 32 (FBXO32) and tripartite motif-containing 63 (TRIM63), which encodes muscle RING-finger protein 1 (MuRF1) (64). The aforementioned study demonstrated the potential of EV-based muscle delivery but should be distinguished from direct evidence of endogenous SkM-EV function. Overall, SkM-EVs and SkM-EV-inspired delivery approaches may regulate SkM remodeling through effects on myogenic cells, fibroblasts and atrophy-associated pathways, although most findings remain preclinical and require validation in human SkM disorder.

Disease-conditioned SkM-EV signaling in impaired muscle homeostasis. The biological effects of SkM-EVs are context-dependent and should not be interpreted as intrinsically beneficial or harmful. Under inflammatory, lipotoxic, glucocorticoid-exposed or atrophic conditions, SkM-EVs may carry disease-conditioned cargo molecules that impair myogenic differentiation, reinforce catabolic signaling and disrupt muscle homeostasis (65). In C2C12-based inflammatory models, myotube-derived EVs originally described as exosome-like vesicles decrease levels of myogenic regulators such as MyoD and myogenin and alter atrophy-associated signaling involving JNK, AMPK, p38 MAPK and mTOR-associated pathways (66,67). Similarly, in glucocorticoid-induced muscle atrophy models, enhanced extracellular export of miR-23a decreases intracellular calcineurin/nuclear factor of activated T cells (NFAT) signaling and facilitates the induction of the atrophy-associated genes FBXO32/atrogin-1 and TRIM63/MuRF1 (68-70). Lipotoxic stress associated with insulin resistance can alter SkM-EV release and cargo composition, linking metabolic stress with impaired myoblast homeostasis (71).

Disease-associated cargo molecules and signaling pathways may amplify muscle dysfunction. MSTN, a negative regulator of SkM growth, is associated with reduced muscle mass and myofiber size in experimental models (72-74). However, MSTN should not be interpreted as a universal SkM-EV cargo molecule unless vesicle-specific localization or enrichment has been experimentally demonstrated. MSTN-associated signaling may represent a catabolic pathway that intersects with disease-conditioned EV responses in SkM (65,73,75). In addition, C2C12 myoblast-derived EVs carrying miR-224 modulate macrophage polarization, suggesting that disease-conditioned SkM-EVs may influence muscle repair indirectly by reshaping the inflammatory microenvironment (67). These findings indicate that disease-conditioned SkM-EVs may propagate inflammatory, catabolic or anti-myogenic programs within SkM.

SkM-EV effects on SkM are better understood as a disease-adaptive conditioned continuum. EVs released under physiological or regenerative conditions may coordinate myoblast differentiation, satellite cell activation, extracellular matrix remodeling and membrane repair, whereas EVs released under inflammatory, lipotoxic, glucocorticoid-exposed or atrophic conditions reinforce catabolic or anti-myogenic programs (76). Thus, the biological direction of SkM-EV signaling depends on donor muscle status, cargo composition, recipient cell identity and disease stage rather than vesicle origin alone.

Extension of local muscle remodeling to muscle-bone crosstalk. Because SkM and bone constitute an integrated locomotor and metabolic unit, SkM-EV signaling may transmit signals associated with muscle contraction, metabolism, inflammation, aging and regeneration to bone-resident cells (77-79). Thus, muscle-bone EV communication can be viewed as a systemic extension of intramuscular remodeling rather than an isolated organ-specific process.

BMSCs are key recipient cells in muscle-bone EV communication because of their role in osteoblast-lineage commitment (57,80). In cell-based and preclinical muscle-to-bone EV studies, C2C12-derived EVs carrying paired related homeobox 2 (Prrx2) transcriptionally upregulate long non-coding RNA MIR22 host gene (MIR22HG), thereby enhancing yes-associated protein 1 (YAP1)-related signaling, alkaline phosphatase activity and osteogenic differentiation in BMSCs (56,57,79). Ma *et al* (57) demonstrated that SkM-EVs deliver glycolytic enzymes, including lactate dehydrogenase A, to enhance BMSC glycolytic flux and upregulate osteogenic markers such as RUNX2 and osterix, revealing a metabolic mechanism of muscle-bone coupling.

Osteoblasts and osteocytes may respond to SkM-EV-associated signals and soluble muscle-derived factors that intersect with EV-associated pathways. Xu *et al* (81) reported that C2C12 myoblast-derived EV miR-27a-3p promotes osteogenic differentiation of MC3T3-E1 cells by suppressing secreted frizzled-related protein 1 (SFRP1) and activating Wnt/β-catenin signaling. When vesicular enrichment has been demonstrated, EV signaling associated with fibronectin type III domain-containing protein 5 (FNDC5)/irisin-related EV signaling may promote osteoblast proliferation and inhibit ferroptosis through caveolin-1-mediated uptake and p38 MAPK/Nrf2 activation (82). Complementary soluble irisin-associated evidence indicates that irisin decreases apoptosis in MLO-Y4 osteocyte-like cells through integrin αV receptor-mediated ERK1/2 signaling (83). These findings suggest functional convergence between vesicle-associated signals and soluble myokine pathways, but vesicular association should be interpreted according to the evidence provided in each study.

SkM-EV-associated signals and soluble muscle-derived endocrine factors influence osteoclasts and chondrocytes. Takafuji *et al* (84) showed that EVs derived from C2C12 myoblasts and myotubes suppress osteoclast markers, including tartrate-resistant acid phosphatase (TRAP) and cathepsin K (CTSK), by inhibiting NFATc1 nuclear translocation. Soluble irisin-associated studies suggest that muscle-derived endocrine signals can regulate osteoclast progenitor metabolism through mitochondrial respiratory chain complex IV activity and reactive oxygen species production (85,86). In experimental osteoarthritis models, irisin-related signaling inhibits NF-κB nuclear translocation, increases type II collagen

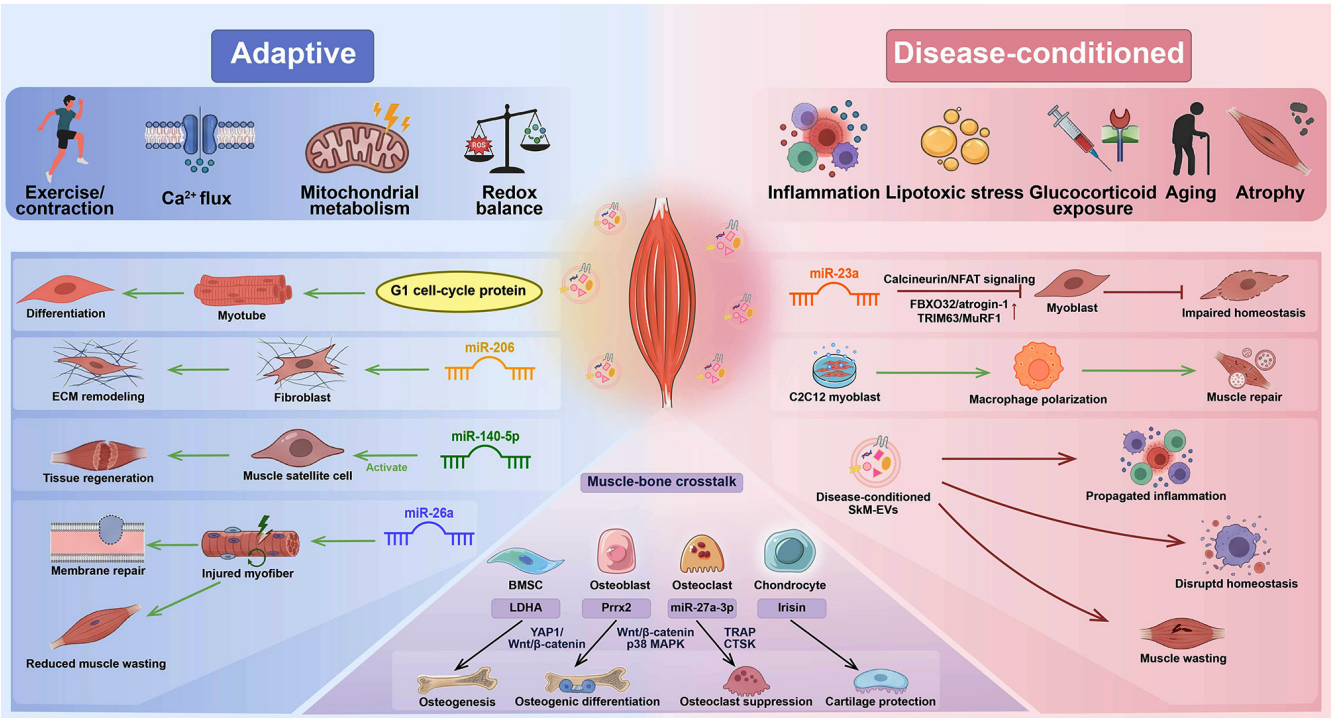

Figure 2. Context-dependent SkM-EV signaling in SkM remodeling and muscle-bone crosstalk. Under exercise/contraction, Ca^{2+} flux, mitochondrial metabolism and redox balance, SkM-EV-associated cargo is associated with myogenic differentiation, extracellular matrix remodeling, satellite cell activation, membrane repair and reduced wasting. Muscle-bone crosstalk involves representative regulatory axes linked to BMSC osteogenesis, osteoblast activity, osteoclast suppression and cartilage protection. Under inflammation, lipotoxic stress, glucocorticoid exposure, aging or atrophy, disease-conditioned SkM-EVs impair myoblast homeostasis, alter macrophage responses, propagate inflammation and promote muscle wasting. SkM-EV, skeletal muscle-derived extracellular vesicle; BMSC, bone marrow mesenchymal stem cell; miR, microRNA; LDHA, lactate dehydrogenase A; ECM, extracellular matrix; Prrx2, paired related homeobox 2; TRAP, tartrate-resistant acid phosphatase; CTSK, cathepsin K; NFAT, nuclear factor of activated T cells; FBXO32, F-box protein 32; TRIM63, tripartite motif-containing 63; MuRF1, muscle RING-finger protein 1.

synthesis and decreases levels of catabolic mediators such as MMP-13 and ADAMTS-5 (87-89). Collectively, these findings support the potential contribution of SkM-EVs and associated muscle-derived signals to skeletal cell biology, with relevance to metabolic bone disorders such as osteoporosis and osteoarthritis. However, most evidence remains preclinical and soluble myokine effects should not be interpreted as direct SkM-EV mechanisms without vesicle-specific validation (Fig. 2).

Shared molecular nodes linking muscle regeneration and bone remodeling. Shared molecular nodes may explain why intramuscular EV effects and muscle-bone crosstalk are mechanistically connected. Canonical myomiRs, including miR-1, miR-133a and miR-206, together with muscle-derived EV-associated miRs such as miR-27a-3p, may regulate myogenic differentiation and osteogenic signaling in a recipient cell-dependent manner (81). In parallel, metabolic enzymes reported in SkM-EVs, such as lactate dehydrogenase A, provide a metabolic link between muscle activity and osteoblast-lineage commitment (57). Mechanotransduction-associated pathways, particularly Hippo/YAP1 signaling, may connect mechanical loading, muscle contraction and osteogenic differentiation (90,91). In addition, soluble myokine pathways that functionally intersect with EV-mediated signaling, including irisin-, IL-6- and IGF-1-associated signaling (92,93), may link exercise adaptation with skeletal remodeling. Conversely, disease-associated cargo molecules or signaling pathways associated with aging, inflammation, myostatin activity or muscle atrophy may transmit catabolic, inflammatory or senescence-associated signals from muscle to bone (94). Therefore, SkM-EVs may be viewed as context-dependent mediators that integrate regenerative, metabolic and degenerative signals across the musculoskeletal system. This framework avoids an artificial separation between local muscle remodeling and muscle-bone crosstalk and supports a more integrated interpretation of SkM-EV biology in degenerative musculoskeletal disease.

4. SkM-EVs in neuromuscular and multi-organ communication

Beyond local effects in SkM and their contribution to musculoskeletal crosstalk, SkM-EVs participate in broader neuromuscular and systemic communication involving the nervous, cardiovascular, renal and immune systems. Signals from fluorescently labeled SkM-EVs have been detected in tissue after exogenous systemic administration in mice, including the brain, heart, pancreas, liver, spleen, lung and gastrointestinal tract (71). However, fluorescence biodistribution data should be interpreted cautiously because tissue accumulation may be influenced by labeling strategy, vesicle source, administration route, dose, sampling time and uptake by the mononuclear phagocyte system (95,96). These findings suggest potential systemic tissue exposure but do not establish endogenous SkM-EV-mediated communication or functional cargo delivery.

SkM-EVs in the neuromuscular axis. SkM-EVs participate in neuromuscular communication by linking SkM with motor neurons, glial cells and the central nervous system (97). SkM is not only an effector tissue under neural control but also a secretory tissue that releases vesicular and soluble signals. These signals influence neural adaptation, motor neuron survival, neurite outgrowth and neuromuscular junction-related remodeling, although most available evidence remains preclinical or cell-based (98,99).

Exercise-induced muscle-derived factors are associated with brain adaptation and neuroprotection. Klein *et al* (100) reported that treadmill training in adult male rats induces SkM-derived factors, including BDNF, which may contribute to neurogenic and gliogenic responses in selected brain regions. Voss *et al* (101) linked aerobic exercise with BDNF-related signaling, brain structural adaptation and cognitive outcomes. Other muscle-associated factors, including IL-6, irisin, FGF21 and IGF-1, are implicated in muscle-brain communication through endocrine or blood-brain barrier-associated mechanisms (102-104). These soluble myokine pathways may intersect with SkM-EV-mediated signaling while representing mechanistically distinct routes of muscle-brain communication.

SkM-EVs affect motor neuron survival and reinnervation. Madison *et al* (105) showed that EVs released from mature differentiated C2C12 cells increase the survival and neurite outgrowth of NSC-34 motor neuron-like cells *in vitro*. In denervation models, muscle-derived EVs support motor neuron regeneration and α-sarcoglycan-positive muscle EVs are detected near muscle branches and neuromuscular junctions following femoral nerve ligation (99,106,107). These findings suggest a role for muscle-derived vesicles in muscle-motor neuron communication and post-denervation reinnervation.

Preclinical evidence suggests that muscle-derived soluble and vesicular signals participate in neural injury responses (108). Irisin-related signaling has been associated with decreased infarct volume, attenuated neuroinflammation and improved neurological outcomes, partly through ERK1/2 and Akt activation (109). Vesicular preparations derived from engineered neuromuscular tissue models have shown enrichment of neurotrophic and myokine-associated signals, including irisin, IL-6, IL-15 and BDNF-associated components, together with regulatory miRs such as let-7 family members and miR-9-5p (36,97). These findings suggest muscle-derived signals may influence neural repair and injury adaptation, although the current evidence remains largely experimental.

Disease-conditioned muscle EVs may contribute to neural dysfunction. SkM-EV-associated miR-29b-3p is linked to aquaporin 4 (AQP4)-mediated water transport and brain edema. It is also associated with impaired neuronal differentiation-related signaling involving FOS proto-oncogene, AP-1 transcription factor subunit (c-FOS), laminin subunit gamma 1 (LAMC1), Ras-like without CAAX 1 (RIT1) and B-cell lymphoma 2 (BCL-2) (110,111). By contrast, exercise-associated SkM-EV miR-484 may protect against brain injury by targeting ACSL4 and decreasing ferroptosis-related damage (108). Together, these findings indicate that the neural effects of SkM-EVs depend on donor muscle status, cargo composition and disease context (Fig. 3A).

Metabolic disease may reshape neuromuscular EV signaling. Diabetes mellitus (DM), characterized by persistent hyperglycemia and insulin resistance, is typically associated with cognitive impairment and peripheral neuropathy (112). Aberrant miR profiles in muscle-associated EVs are linked to metabolic dysfunction and may contribute to neural complications in DM (113). However, the direct contribution of SkM-EVs to diabetic central nervous system pathology remains insufficiently defined.

Overall, SkM-EVs may contribute to neuromuscular communication through vesicle-associated miRs and other exercise- or disease-responsive cargo molecules. Evidence supports potential roles in motor neuron survival, neuronal differentiation, neuroprotection and disease-conditioned neural dysfunction, but most mechanistic findings remain preclinical (105,111).

SkM-EVs and muscle-heart communication. The heart depends on coordinated intercellular and interorgan communication to maintain contractile function and adapt to stress. SkM-EVs participate in muscle-heart communication through miR delivery, protein-associated signaling and exercise-responsive vesicular pathways (114). Signals from fluorescently labeled SkM-EVs have been detected in myocardial tissue following exogenous systemic administration in mice (115). However, cardiac accumulation should be interpreted cautiously unless supported by quantitative biodistribution, intact vesicle tracking and source validation approaches.

Striated muscle-associated and cardiac-enriched miRs have been linked to cardiac injury and remodeling (116,117). Myocardial injury is associated with altered expression of miR-499, miR-1, miR-133 and miR-208 (118). Clinical studies have reported altered circulating or serum levels of miR-1, miR-133 and miR-208 in heart failure and acute myocardial infarction (119-121). These findings support the biomarker potential of striated muscle- or cardiac-associated miRs, but do not establish SkM-derived EV origin.

Preclinical studies suggest that muscle disease-associated vesicular signals, exercise-induced circulating EVs and engineered EV systems influence cardiac remodeling through distinct mechanisms (122,123). In muscular dystrophy, elevated SkM-EV miR-16-5p is associated with impaired cardiomyocyte autophagic flux by targeting sestrin 1, suggesting a potential connection between SkM pathology and cardiac dysfunction (124). Exercise-induced circulating EVs have shown cardioprotective effects in oxidative stress and cardiac ischemia/reperfusion injury models, including enhanced antioxidant defenses, activation of ERK1/2 and HSP27 signaling, decreased cardiomyocyte apoptosis and delivery of exercise-responsive exosomal miRs such as miR-342-5p (122,125). In addition, engineered EVs loaded with miR-148a improve cardiac function, increase dystrophin expression and decrease myocardial fibrosis in a preclinical Duchenne muscular dystrophy model (123) (Fig. 3B).

Taken together, SkM-EVs, exercise-induced circulating EVs and EV-inspired engineered systems may contribute to muscle-heart communication and cardiac stress adaptation (114,122,126). However, circulating myomiRs should be interpreted as striated muscle- or cardiac-associated biomarkers rather than definitive SkM-derived EV markers.

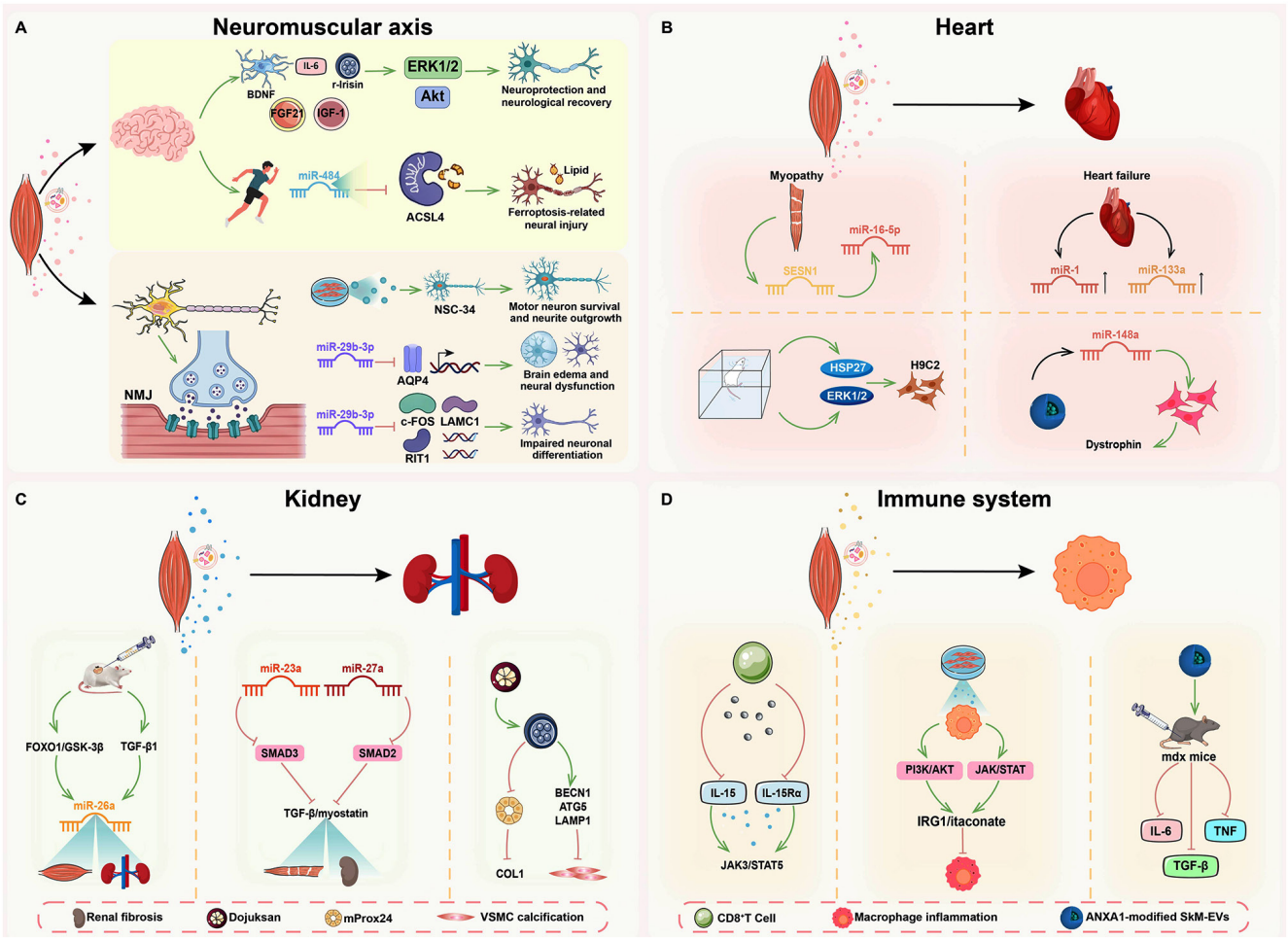

Figure 3. SkM-EV-associated neuromuscular and multi-organ communication. (A) SkM-EVs and muscle-derived signals are involved in neuromuscular communication involving the brain, motor neurons and NMJs. Representative pathways include ERK1/2/Akt-associated neuroprotection, miR-484/ACSL4 signaling and miR-29b-3p-associated neural injury response. (B) SkM-, exercise-induced circulating and engineered EVs are involved in muscle-heart communication through miR-16-5p/SESN1, ERK1/2/HSP27 and miR-148a/dystrophin-associated pathways. (C) In muscle-kidney communication, SkM-EV-associated miR-26a, miR-23a and miR-27a are associated with FOXO1/GSK-3β, TGF-β/myostatin and SMAD2/3-related signaling, whereas irisin-associated autophagy signaling is a complementary muscle-derived pathway. (D) Muscle-derived IL-15/IL-15Rα signaling, SkM-EV-associated macrophage pathways and ANXA1-modified SkM-EVs are involved in muscle-immune communication and dystrophic muscle inflammation. SkM-EV, skeletal muscle-derived extracellular vesicle; miR, microRNA; SESN1, sestrin 1; HSP, heat shock protein; GSK-3β, glycogen synthase kinase-3β; ANXA1, annexin A1; BDNF, brain-derived neurotrophic factor; r-, recombinant; FGF, fibroblast growth factor; IGF, insulin-like growth factor; AQP4, aquaporin 4; LAMC1, laminin subunit γ1; RIT1, Ras-like without CAAX 1; NMJ, neuromuscular junction; mProx, mouse proximal tubular epithelial cell line; BECN1, beclin 1; LAMP1, lysosome-associated membrane protein 1; COL, collagen; VSMC, vascular smooth muscle cell; IRG1, immunoresponsive gene 1; EX, exercise.

Future studies should clarify source attribution, cardiac biodistribution, dosing regimens that achieve biologically effective cardiac exposure, long-term safety and mechanism-based potency and determine whether cardioprotective effects are mediated by intact vesicles, specific cargo molecules or co-isolated non-vesicular factors.

SkM-EVs and muscle-kidney communication. The kidney is susceptible to fibrosis, metabolic stress and chronic inflammation during chronic degenerative or metabolic disease (127,128). SkM-EVs and muscle-derived EV-associated miR signals are implicated in muscle-kidney communication, particularly in preclinical models of CKD, unilateral ureteral obstruction (UUO) and diabetic nephropathy (64,129-131).

Muscle-derived EV-associated miRs influence both muscle wasting and renal fibrosis. SkM-derived miR-26a delivered in EVs originally described as exosome-like vesicles increase miR-26a levels in muscle and kidney tissues in UUO mice, attenuates muscle atrophy through the forkhead box O1 (FoxO1)/glycogen synthase kinase-3β (GSK-3β) pathway and decrease renal fibrosis by down-regulating connective tissue growth factor and TGF-β1 expression (130). In preclinical models, muscle-associated EV-mediated delivery of miR-23a and miR-27a has been shown to suppress TGF-β/myostatin signaling through Smad2/3 regulation, thereby ameliorating diabetes-associated muscle atrophy and renal complications, while related preclinical evidence also supports miR-23a/27a as anti-atrophy regulators in muscle wasting (131,132). Although not a direct EV-based approach, localized adeno-associated virus-mediated miR-29a (AAV-miR-29a) delivery to SkM decreases expression of renal fibrosis-related proteins, including Yin Yang 1 transcription factor (YY1), TGF-β and collagen type I alpha 1 chain (COL1A1), suggesting that muscle-derived miR-29a may contribute to anti-fibrotic muscle-kidney signaling (133).

Soluble myokine pathways provide complementary evidence for muscle-kidney communication. Irisin-associated signaling has been associated with anti-inflammatory, antioxidant and anti-apoptotic effects in CKD-associated renal injury (134). Activation of the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α)/FNDC5 pathway increase irisin secretion and suppress TGF-β-induced collagen 1 expression in renal tubular epithelial cells (135). Irisin mitigates renal fibrosis and vascular calcification by activating autophagy-associated pathways involving beclin 1, autophagy-related 5 and lysosomal-associated membrane protein 1, and by suppressing NLRP3 inflammasome activation (136). These findings support a role for muscle-derived endocrine signals in renal protection (Fig. 3C).

Collectively, preclinical evidence suggests muscle-derived EV-associated miRs, including miR-26a, miR-23a and miR-27a, together with muscle-directed miR-29a expression models, may contribute to muscle-kidney communication and renal fibrosis modulation (130,131,133). Direct human evidence remains limited and is mainly derived from circulating, plasma or urinary EV-miRNA profiling studies in diabetic nephropathy or KD, rather than direct SkM-EV source-tracing or renal delivery studies (137-139). Future studies should verify SkM-EV source, renal biodistribution, cargo specificity and causal mechanisms before SkM-EV-based renal interventions advance toward translational development.

SkM-EVs and muscle-immune communication. Immune regulation depends on coordinated signaling between immune cells, stromal tissues and soluble or vesicular mediators. As an immunometabolic tissue, SkM shapes immune responses through cytokines, metabolites and EV-associated signals (140). SkM-EVs contribute to muscle-immune communication by modulating macrophage polarization and inflammatory signaling under specific pathological conditions, and SkM-derived secreted signals may influence adaptive immune responses during chronic inflammatory stress (67,141,142).

SkM-derived signals are implicated in systemic immune regulation during chronic inflammatory stress (142). Chronic infection-induced $CD8^+$ T cell exhaustion is associated with muscle atrophy (143). In a lymphocytic choriomeningitis virus model, muscle-derived IL-15/IL-15Rα signaling activates JAK3/STAT5 and preserves antiviral $CD8^+$ T cell function. This response is accompanied by remodeling of erythronate-associated metabolic activity and altered FNDC5/irisin-associated myokine signaling, suggesting chronic inflammatory stress reshapes both metabolic and endocrine features of SkM-derived immune regulation (142). Although this evidence is not EV-specific, it supports the hypothesis that SkM influences immune function through secreted molecular signals.

More direct evidence for SkM-EV-mediated immunomodulation comes from macrophage-associated studies (67,141). Myotube-derived EVs attenuate macrophage inflammatory responses through PI3K/Akt and JAK/STAT pathway activation, immunoresponsive gene 1-itaconate upregulation and enrichment of anti-inflammatory miRs such as miR-206-3p and miR-378a-3p (141). These findings suggest SkM-EVs regulate the inflammatory tone of the regenerating muscle microenvironment by influencing macrophage activation states.

Engineering strategies may enhance the immunomodulatory potential of SkM-EVs. Magnetically guided annexin A1 (ANXA1)-modified SkM-EVs delivered into the tibialis anterior muscle of mdx mice suppress levels of inflammatory and fibrosis-associated mediators, including tumor necrosis factor, IL-6 and TGF-β (144). This preclinical evidence suggests engineered SkM-EVs may serve as candidate immunomodulatory delivery systems for dystrophic muscle inflammation, although targeting efficiency, biodistribution, quality control and long-term safety remain to be established (Fig. 3D).

Together, SkM-EVs may participate in muscle-immune communication by modulating macrophage responses, inflammatory signaling and immunometabolic adaptation. However, most findings remain preclinical and SkM-EV-based immunomodulatory strategies require validation in clinically relevant models of inflammatory and degenerative muscle disease, especially with respect to EV source attribution, immune cell targeting and cargo-dependent mechanisms.

5. Context-dependent remodeling of SkM-EV release and cargo profiles

SkM-EV release and cargo profiles vary across physiological, pathological and experimental contexts, including exercise, aging, alcohol exposure, metabolic stress, exogenous EV administration and EV-engineering strategies (Table II) (145). These conditions reshape vesicle output and biological activity, thereby influencing local SkM remodeling and interorgan communication.

Exercise-induced remodeling of SkM-EV release and cargo profiles. Mechanical loading, metabolic stress and contraction-induced signaling during exercise remodel circulating EV abundance and cargo profiles. Small EVs or EVs with exosome-like dimensions increase rapidly in the circulation during the aerobic phase of exercise, suggesting physical activity can acutely reshape EV communication (146). However, the relative contribution of SkM to this circulating EV pool remains uncertain because exercise may simultaneously mobilize EVs from endothelial cells, platelets, immune cells and adipose and other metabolically active tissue (147).

Exercise-induced changes in EV-associated markers and abundance are not fully consistent across studies: Altered expression of Alix following acute exercise suggests potential changes in EV-associated components of the endosomal sorting complex required for transport machinery, whereas other studies have found no significant changes in Alix, TSG101 or CD63 after aerobic or combined aerobic and resistance exercise (148,149). Similarly, endurance exercise studies have reported divergent effects on circulating EV abundance, ranging from no significant change following 1 h endurance exercise to increased EV levels after 30 min of moderate-intensity exercise (150,151). These discrepancies may reflect differences in participant characteristics, exercise modality, sampling time, biofluid source, EV isolation method and normalization strategy.

Exercise-responsive EV cargo molecules may participate in adaptive remodeling and anti-atrophic signaling. In a preclinical CKD model, exercise-associated miR-23a and

Table II. Context-dependent remodeling of muscle-associated EV release and cargo profiles.

Context	EV alteration	Functional implication	Evidence level	(Refs.)
Exercise	Increasedcirculating small EV levels; increased plasma EV ACTN4/ADAM10/ ALIX/CD81 signaling; exercise-associated miR-23a/miR-27a	Adaptive or anti-atrophic signaling	Human/animal	(132,146, 148-152)
Senescent FAP-derived EVs	Increased let-7 family miR expression	Decreased satellite cell support	Animal	(145)
Exercise modality	Up/downhill running-associated changes in miR-1/miR-133a/miR-499 profiles	Muscle recruitment pattern-dependent myomiR remodeling	Clinical	(153,154)
Mechanical loading	Mechanical strain-induced EV release; strain-responsive EV-associated miR-146b-5p/miR-210-3p cargo	Mechanotransduction-associated EV remodeling	Cell	(154,155)
Aging	Age-associated changes inmiR-1/miR-133a/ miR-133b/miR-206;decreased EV-associated miR-1/ miR-206 and ALIX/α-sarcoglycan/syntenin levels	Impaired regenerative niche and sarcopenia-associated communication	Human/cell	(31,159, 160)
Alcohol + SIV stress	Decreased miR-206; increased miR-133a/b	Disrupted myogenic programs	Animal	(163,164, 166)
Plant-derived vesicle-like particles	Increased MYF5/MYOD1/MYOG signaling; activated AMPK/ SIRT1/PGC-1α	Exogenous modulation of myogenic differentiation and metabolic remodeling	Animal	(171,173, 174)
Irisin-associated myokine signaling	Activated PGC-1α/FNDC5/ irisin signaling	Myogenic, metabolic and anti-apoptotic effects	Animal/cell	(175-183)

ACTN4, actinin alpha 4; ADAM10, a disintegrin and metalloproteinase domain-containing protein 10; ALIX, ALG-2-interacting protein X; AMPK, AMP-activated protein kinase; FAP, fibro-adipogenic progenitor; FNDC5, fibronectin type III domain-containing protein 5; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1α; SIRT1, sirtuin 1; SIV, simian immunodeficiency virus; SkM-EV, skeletal muscle-derived extracellular vesicle; myomiR, muscle-enriched microRNA; ALIX, ALG-2-interacting protein X; let-7, lethal-7 family of microRNAs; MYF5, myogenic factor 5; MYOD1, myogenic differentiation 1; MYOG, myogenin.

miR-27a in muscle-associated EV preparations are associated with suppression of Smad2/3 signaling and attenuation of muscle wasting (132). Proteomic profiling of human plasma EVs following acute aerobic exercise also revealed increased levels of ACTN4, ADAM10, Alix and CD81, supporting the hypothesis that exercise can remodel EV cargo profiles involved in tissue communication (152). Nevertheless, plasma EV changes should not be interpreted as direct evidence of SkM-EV release without muscle-specific source validation or tissue-tracing.

Exercise modality, duration and muscle recruitment pattern influence EV cargo output. Up- and downhill running differentially recruit muscle groups and alter myomiR expression patterns, including miR-1, miR-133a and miR-499 (153). In *ex vivo* C2C12 myoblast mechanical-strain models, mechanical loading modulates EV release and EV-associated miR cargo, including miR-146b-5p and miR-210-3p, with pathway analyses implicating mechanotransduction- and inflammatory signaling-associated pathways involving YAP/TAZ and TRAF6/NF-κB signaling (154,155). Collectively, exercise-responsive EV remodeling, including putative SkM-EV contributions, may depend on exercise intensity, duration, muscle recruitment pattern, sampling time, EV isolation strategy and analytical platform (Fig. 4A).

Aging-associated changes in SkM-EV cargo profiles. Aging is characterized by mitochondrial dysfunction, impaired proteostasis, chronic low-grade inflammation and progressive decline in tissue repair capacity (156). In SkM, these changes are associated with sarcopenia, decreased regenerative potential and impaired muscle-organ communication (157). Aging-associated remodeling of SkM-EV cargo profiles may contribute to this process by altering the transfer of myomiRs, senescence-associated miRs and EV-associated proteins between SkM and recipient tissue (158).

Aging-related changes in myomiRs are inconsistent across tissue- and EV-based studies (31,159). SkM analyses have reported age-dependent alterations in canonical myomiRs, including miR-1, miR-133a, miR-133b and miR-206, whereas EV-level studies suggest that selected myomiRs, particularly miR-1 and miR-206, may decline in aged muscle-associated EVs (31,160). These changes may be accompanied by decreased

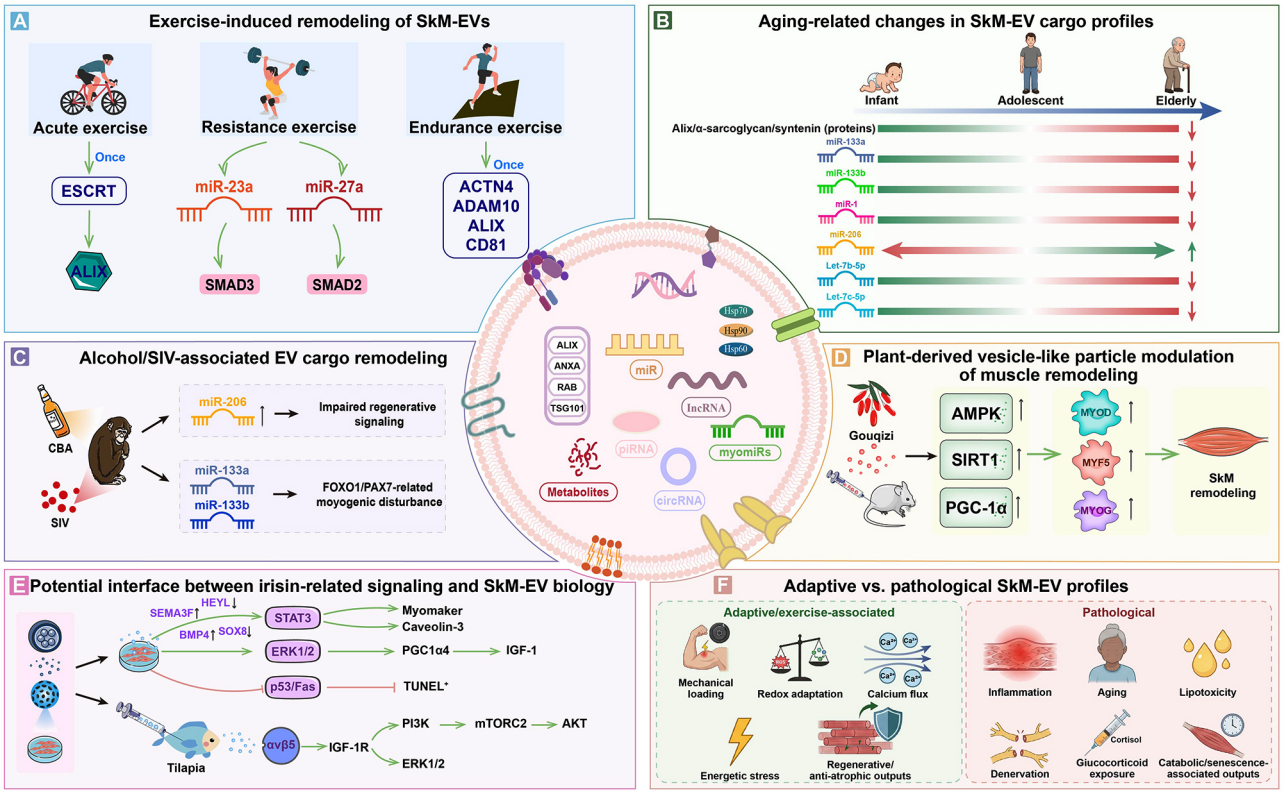

Figure 4. Physiological and pathological factors shaping SkM-EV release and cargo profiles. (A) Exercise remodels circulating or muscle-related EV cargos, including ALIX, ACTN4, ADAM10, CD81 and miR-23/miR-27-related SMAD signaling. (B) Aging alters muscle-associated EV proteins, myomiRs and stromal EV-associated let-7 family members. (C) Alcohol/SIV-associated stress changes miR-206 and miR-133a/b-associated myogenic regulatory profiles. (D) Plant-derived vesicle-like particles modulate AMPK/SIRT1/PGC-1α- and MYOD1/MYF5/MYOG-associated pathways. (E) Irisin-related signaling is a potential interface with SkM-EV biology. (F) Adaptive and disease-conditioned SkM-EV profiles are shaped by loading, redox adaptation, metabolic stress, inflammation, aging and catabolic stimuli. SkM-EV, skeletal muscle-derived extracellular vesicle; ALIX, ALG-2-interacting protein X; myomiR, muscle-enriched microRNA; let-7, lethal-7; SIV, simian immunodeficiency virus; MYOD1, myogenic differentiation 1; MYF5, myogenic factor 5; MYOG, myogenin; ESCRT, endosomal sorting complex required for transport; CBA, chronic binge alcohol administration; ANXA1, annexin A1; RAB, Ras-related protein Rab; TSG101, tumor susceptibility gene 101 protein; circRNA, circular RNA; lncRNA, long non-coding RNA; piRNA, PIWI-interacting RNA; HEYL, HES-related family bHLH transcription factor with YRPW motif-like; SEMA3F, semaphorin 3F; BMP4, bone morphogenetic protein 4; αvβ5, integrin αvβ5; IGF-1R, insulin-like growth factor 1 receptor.

levels of EV-associated proteins and muscle-associated markers, such as Alix, α-sarcoglycan and syntenin, in aging myotube cultures (159). The divergent findings may reflect differences in species, age range, muscle type, fiber composition, sample source, EV isolation method and normalization strategy.

Aging may remodel EV-mediated communication within the SkM niche through non-myogenic stromal cells. Fibro-adipogenic progenitors, which regulate muscle regeneration and satellite cell function, release EVs enriched in senescence-associated miRs, including let-7 family members (145). These stromal EVs may contribute to impaired regenerative signaling during aging. Although they should be distinguished from myofiber- or myotube-derived SkM-EVs, they are relevant to the aged SkM EV microenvironment (Fig. 4B).

Overall, aging may remodel the miR and protein cargo profiles of muscle-associated EVs, but their source specificity and causal roles in sarcopenia remain insufficiently defined. Further studies are needed to determine whether aging-associated SkM-EV signatures represent SkM-intrinsic remodeling or broader systemic changes involving immune, vascular and metabolic tissue.

Alcohol-associated remodeling of muscle-related EV cargo profiles. Alcohol exposure can disrupt SkM homeostasis by inducing metabolic dysfunction, mitochondrial impairment, inflammation, impaired regeneration and fibrosis (161). Chronic heavy alcohol intake is associated with alcohol-induced muscle disease and muscle wasting, but the contribution of SkM-EV remodeling to this process remains incompletely defined (162). Evidence from chronic binge alcohol-administered simian immunodeficiency virus (SIV)-infected macaques and alcoholic liver disease-associated EV studies suggests that alcohol-associated EV changes may be influenced by exposure pattern, SIV-associated stress, host metabolic or liver disease status and tissue source (163-165).

In chronic binge alcohol exposure models combined with SIV infection, muscle-associated EV miR profiles are notably remodeled (163,164). Plasma EV-associated miR-206 levels, in vesicles originally described as exosomes, are decreased under combined alcohol and SIV exposure compared with alcohol exposure alone (166). In SkM EV preparations from SIV-infected rhesus monkeys, chronic binge alcohol exposure increases EV-associated miR-133a and miR-133b levels, with predicted or reported targeting of FOXO1 and paired box 7, regulators involved in myogenic differentiation and satellite

cell biology (163). Additional studies also report reduced SkM-associated myomiRs, including miR-206, in skeletal muscle, plasma or myotube-derived EVs under combined chronic binge alcohol and SIV stress (164,166). These findings suggest that alcohol exposure and SIV-associated stress may jointly remodel muscle-associated EV miR profiles and impair myogenic regulation (Fig. 4C).

Overall, alcohol-associated EV remodeling may involve downregulation of regeneration-associated miRs, such as miR-206, and dysregulation of miR-133a/133b-related pathways. However, most evidence comes from animal or non-human primate models under combined pathological exposures, which do not fully separate the effects of alcohol from SIV-associated inflammatory or metabolic stress. Future studies should determine whether alcohol alone directly alters SkM-EV release, cargo loading and recipient cell responses in human SkM and whether these changes contribute causally to alcohol-induced muscle disease.

Exogenous plant-derived vesicle-like particles in SkM remodeling. Plant-derived EVs or vesicle-like nanoparticles are membrane-enriched particles obtained from plant tissue or cells and may contain bioactive lipids, proteins and nucleic acids (167). Because of their relative biocompatibility, low immunogenic potential and cargo-protective capacity, they have attracted interest as orally administrable or injectable vesicle-like delivery systems (168-170). However, these particles should not be regarded as direct regulators of endogenous SkM-EV release or cargo sorting unless this has been experimentally demonstrated. Their relevance lies primarily in their potential to modulate SkM remodeling through exogenous vesicle-based delivery.

Plant-derived vesicle-like particles have shown potential effects on SkM remodeling in experimental models (171,172). Gouqizi-derived nanovesicles delivered into quadriceps muscle upregulate myogenic regulatory factors, including MYF5, MYOD1 and MYOG, in a muscle atrophy model (171). These effects are associated with polysaccharide- and sphingolipid-enriched vesicular components and activation of AMPK/SIRT1/PGC1α signaling, suggesting potential regulation of mitochondrial biogenesis and myofiber remodeling (171). Other plant-derived vesicle-like particles, including grape- and ginger-derived preparations, modulate oxidative stress-, inflammation- or Nrf2-associated pathways, although their direct effects on SkM atrophy remain less well established (173,174). However, whether these particles reshape endogenous SkM-EV release, cargo composition or recipient cell responses remains unclear (Fig. 4D). Therefore, plant-derived vesicle-like particles should be positioned as exogenous vesicle-based modulators of SkM remodeling rather than established regulators of SkM-EV biology. Future studies should determine whether these particles influence endogenous SkM-EV release, cargo sorting or recipient cell responses and whether plant-animal vesicle communication produces reproducible effects in clinically relevant muscle-degenerative conditions.

Irisin-related myokine signaling and its potential interface with SkM-EVs. Irisin is a cleavage product of FNDC5 and is released by SkM, particularly under exercise-related conditions (175). Through the PGC-1α/FNDC5 axis, irisin participates in metabolic regulation, adipose tissue browning, myogenic adaptation and muscle-bone communication (176,177). However, irisin should be regarded primarily as a soluble myokine-related signal unless vesicular localization, enrichment or functional delivery has been directly demonstrated. Accordingly, its association with SkM-EV release, cargo sorting and EV-mediated delivery remains unresolved.

Irisin-associated signaling is implicated in muscle growth, myogenic differentiation and metabolic adaptation. In MSTN-deficient or recombinant irisin-treated models, irisin is associated with FNDC5 upregulation, AMPK/mTORC1 activation, increased uncoupling protein 1 expression and white adipose browning (178,179). In C2C12 myoblasts and myotubes, recombinant irisin promotes myoblast fusion and myogenic signaling through pathways involving STAT3, myomaker, caveolin-3, Wnt/β-catenin, ERK1/2 and the IGF-1/PGC1α4 pathway (180). These findings support a role for irisin in myogenesis, muscle secretory activity and hypertrophic adaptation, but do not establish irisin as a SkM-EV cargo molecule.

Irisin has also shown protective effects in experimental models of muscle aging and development (180-182). In D-galactose-induced SkM aging models, irisin decreases apoptotic signaling, inhibits the p53/Fas pathway and improves mitochondrial membrane potential (181,183). In a non-mammalian muscle development model, irisin activates ERK1/2 and PI3K/mTORC2/Akt signaling through integrin αvβ5, a heterodimeric receptor composed of αv and β5 integrin subunits, and insulin-like growth factor 1 receptor-associated pathways, thereby influencing mitochondrial function and muscle development (182). These findings support irisin as a multifunctional myokine involved in metabolism, myogenesis and apoptosis resistance, while remaining distinct from vesicle-specific SkM-EV mechanisms (Fig. 4E).

Overall, irisin-associated signaling may interface with SkM-EV biology indirectly by reshaping the exercise-responsive muscle secretome, donor cell metabolic state or muscle-organ communication. Future studies should determine whether irisin directly regulates SkM-EV release, cargo sorting, tissue uptake or biological potency under defined exercise, aging and disease conditions. Such evidence is necessary before irisin-associated pathways can be rationally incorporated into engineered SkM-EV delivery strategies.

Molecular distinction between adaptive and disease-conditioned SkM-EV profiles. Adaptive and disease-conditioned SkM-EV profiles may differ in upstream stimuli, vesicle output, cargo selection, recipient cell responses and biological directionality (184). Exercise-responsive SkM-EVs or muscle-associated EVs are shaped by mechanical loading, contraction-induced Ca^{2+} signaling, energetic stress and redox adaptation (154). These stimuli engage mechanotransduction, AMPK-associated metabolic regulation, YAP/TAZ activity, redox-sensitive pathways and mitochondrial regulatory networks, thereby influencing the selective loading of miRs, proteins and metabolic enzymes into EVs (185). Functionally, exercise-responsive EVs are typically associated with adaptive remodeling, including enhanced

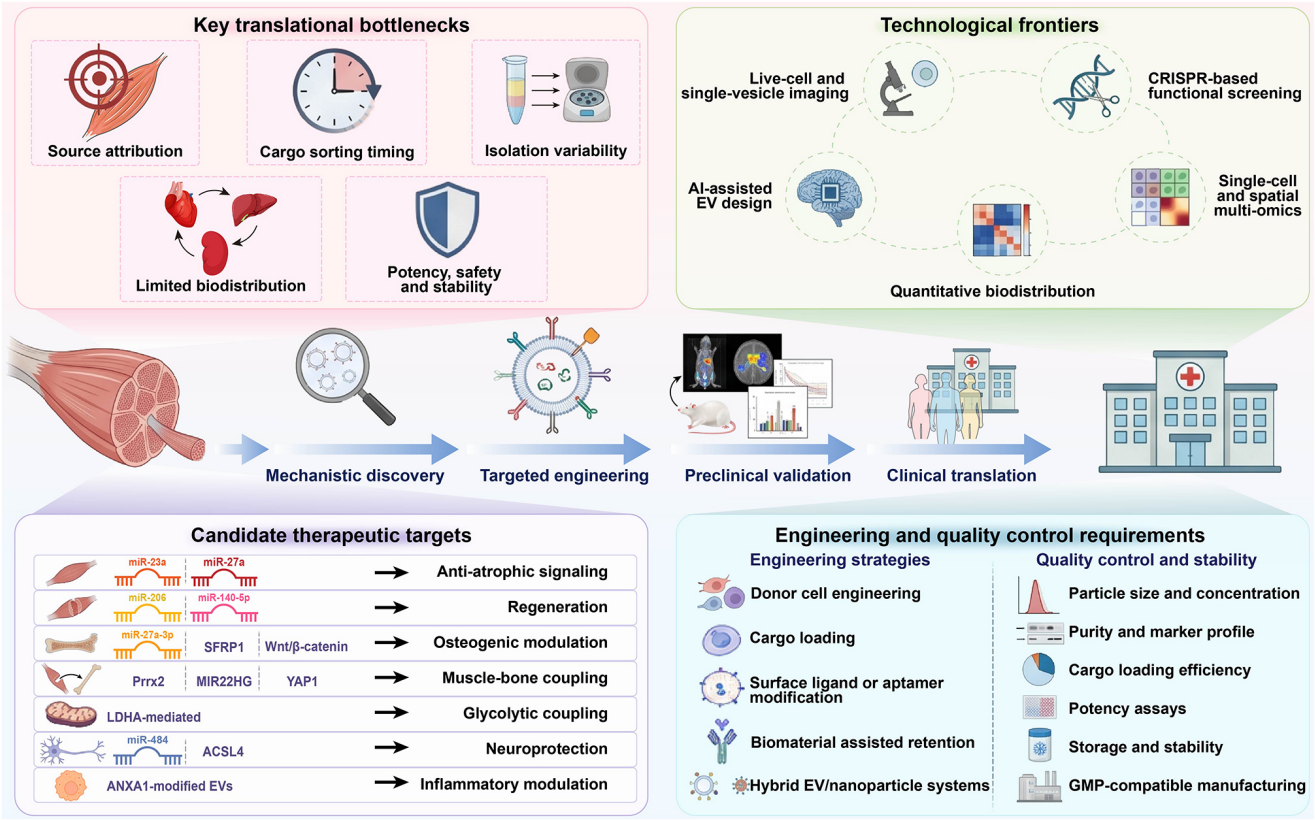

Figure 5. Translational roadmap, key bottlenecks, candidate therapeutic axes, engineering strategies, technological frontiers and quality control requirements for SkM-EV-based therapeutic development. Candidate axes include anti-atrophic, regenerative, osteogenic, glycolytic, neuroprotective and immunomodulatory signaling. Engineering strategies include donor cell engineering, cargo loading, surface ligand or aptamer modification, biomaterial-assisted retention and hybrid EV-nanoparticle systems. Translational requirements include source attribution, standardized isolation and characterization, quantitative biodistribution, potency assays, stability testing, safety evaluation and GMP-compatible manufacturing. SkM-EV, skeletal muscle-derived extracellular vesicle; GMP, good manufacturing practice; CRISPR, clustered regularly interspaced short palindromic repeats; AI, artificial intelligence; miR, microRNA; SFRP1, secreted frizzled-related protein 1; Prrx2, paired related homeobox 2; MIR22HG, MIR22 host gene; LDHA, lactate dehydrogenase A; ANXA1, annexin A1.

myogenic repair, improved metabolic flexibility, decreased atrophy-related signaling and coordinated tissue crosstalk during physiological stress (152).

Disease-conditioned SkM-EV profiles may arise from chronic inflammatory stimulation, lipotoxicity, insulin resistance, glucocorticoid exposure, denervation, aging or muscle atrophy (65,76). These conditions generate EV cargo molecules that reinforce tissue dysfunction rather than adaptive remodeling. Inflammatory myotube-derived vesicles may impair myogenic differentiation and enhance atrophy-associated signaling, whereas lipid-induced insulin resistance can alter muscle EV release and disrupt myoblast homeostasis (71). Aging- or atrophy-associated EV miRs may transmit senescence-associated, catabolic or neuromuscular dysfunction signals to recipient cells (158).

The distinction between adaptive and disease-conditioned SkM-EV profiles is not absolute. SkM-EV output and cargo composition may vary according to exercise modality, intensity, training status, disease stage, muscle fiber composition, sampling time and EV isolation method (186,187). Direct comparison of exercise-responsive and disease-associated EVs under standardized experimental conditions, combined with muscle-specific source attribution and functional validation, is key for defining adaptive vs. disease-conditioned vesicular signatures (Fig. 4F).

6. Therapeutic targets and technological frontiers for SkM-EV-based intervention

Candidate therapeutic targets, engineering strategies and translational considerations for SkM-EV-based intervention are summarized in Fig. 5. Given the largely preclinical status of SkM-EV research, SkM-EV-specific evidence should be distinguished from broader experience in EV-based therapeutic translation.

The translational potential of SkM-EVs depends not only on their proposed roles in interorgan communication, but also on whether disease-relevant cargo molecules, target cell specificity and delivery technologies can be rationally defined and reproducibly validated (188). Recent advances in the EV field, including EV engineering, single-vesicle analysis, quantitative biodistribution, multi-omics profiling and clinical-grade manufacturing, address gaps in SkM-EV research, including source attribution, EV heterogeneity, cargo-function causality, target-organ biodistribution, potency assessment and batch-reproducible production (189,190). These technologies may move the field from descriptive EV cargo profiling toward mechanism-guided, potency-defined and target-oriented preclinical development.

Candidate therapeutic targets in SkM-EV-mediated interorgan communication. Several SkM-EV-associated cargo molecules and signaling axes have been proposed as

candidate therapeutic targets for degenerative and metabolic disease. In SkM, miR-23a and miR-27a may counteract muscle wasting by suppressing Smad2/3-associated catabolic signaling (131,132), whereas miR-206 and miR-140-5p are implicated in extracellular matrix remodeling, satellite cell activation and post-injury muscle regeneration (55,58). In the musculoskeletal system, miR-27a-3p/SFRP1/Wnt/β-catenin and Prrx2/MIR22HG/YAP1 signaling and LDHA-mediated glycolytic coupling represent potential targets linking muscle-derived vesicular signals with osteogenic differentiation and bone remodeling (56,57). Where vesicular enrichment has been demonstrated, FNDC5/irisin-enriched EVs may promote osteoblast proliferation and inhibit ferroptosis through caveolin-1-mediated uptake and downstream p38 MAPK/Nrf2 activation (82). In the neuromuscular axis, exercise-associated SkM-EV miR-484 may suppress neuronal ferroptosis through the ACSL4 pathway (108), whereas ANXA1-modified EVs and anti-inflammatory miR cargo molecules provide candidate strategies for modulating macrophage activation and local inflammatory damage (141,144). These targets should be prioritized according to disease context, target cell type, evidence level and vesicle-specific validation, rather than being treated as universally beneficial or clinically validated EV cargo molecules.

Engineering strategies for targeted SkM-EV and EV-inspired delivery. Engineering strategies may improve the delivery selectivity, cargo consistency and functional reproducibility of SkM-EVs. Donor cell engineering can enrich EVs with defined proteins, miRs or therapeutic RNAs, whereas post-isolation loading can introduce small molecules, nucleic acids or proteins into EV preparations (191). Surface modification strategies, including ligand display, peptide conjugation, aptamer modification and membrane hybridization, may enhance target tissue or target cell enrichment in bone, muscle, neural or inflammatory microenvironments. For example, FNDC5/irisin-enriched small EVs conjugated with a bone-targeting aptamer attenuate ovariectomy-induced osteoporosis-like phenotypes in preclinical mouse models, suggesting myokine-associated vesicular signals can be integrated with tissue-targeting engineering to improve delivery selectivity (192,193). Biomaterial-assisted delivery systems, including injectable hydrogels, scaffolds and magnetic or nanoparticle-based platforms, may improve local retention, protect EV cargo molecules and provide sustained release in muscle injury, osteoarthritis or bone-loss microenvironments (194). However, these engineered systems require source-specific characterization, biodistribution analysis, mechanism-based potency assays, batch reproducibility and safety evaluation before they are considered translationally robust.

Quality control, enabling technologies and translational requirements. Quality control is key for the preclinical and translational development of engineered SkM-EV-based strategies. Compared with unmodified SkM-EVs, engineered SkM-EVs require additional characterization because cargo loading, surface modification or membrane hybridization may alter vesicle composition, apparent identity, purity, biodistribution and potency (195). Key quality attributes should include particle concentration, size distribution, morphology, EV- and SkM-associated and source-appropriate contaminant markers, cargo loading efficiency, surface modification density, residual free cargo, unincorporated ligands, residual loading reagents, sterility, endotoxin levels, mycoplasma contamination and cargo-dependent potency (126,196). Stability testing should determine whether storage temperature and duration, buffer composition, freeze-thaw cycles, lyophilization or cryoprotectants affect vesicle aggregation, membrane integrity, cargo retention and biological activity (195).

Technological advances may address limitations in SkM-EV research. Single-vesicle imaging and single-particle analysis improve the resolution of EV heterogeneity and distinguish muscle-derived vesicle subpopulations from co-isolated particles or EVs derived from other tissue (197). Integrated proteomic, transcriptomic, lipidomic and metabolomic profiling define context-specific EV signatures associated with exercise, aging, inflammation, denervation and metabolic disease (198). Quantitative *in vivo* biodistribution approaches are needed to determine whether unmodified or engineered SkM-EVs reach bone, brain, kidney, heart or immune tissue at biologically relevant levels (199). Microfluidic separation, high-sensitivity biosensing and standardized potency assays may improve reproducibility and support mechanism-based preclinical development (200). Good manufacturing practice (GMP)-compatible manufacturing requires strict control of donor cell source, batch consistency, purity, sterility, cargo stability and biological potency (201). Artificial intelligence and machine learning may assist in prioritizing donor cells, therapeutic cargo molecules, targeting ligands and disease-specific EV signatures (188), but these predictions require experimental validation before they guide SkM-EV engineering or therapeutic design.

Clinical translation status and selected EV-based therapeutic trials. Although SkM-EVs and SkM-EV-inspired strategies have shown candidate therapeutic effects in preclinical models of muscle wasting, osteoporosis, osteoarthritis, renal fibrosis and neural injury, SkM-EV-specific therapy has not reached a mature clinical stage. Most available evidence is derived from SkM cell cultures, rodent disease models, exercise-conditioned EV profiling or engineered EV delivery experiments (7,126). To date, therapeutic EV studies have primarily focused on EVs derived from MSCs, umbilical cord-derived MSCs (UC-MSCs), BMSCs or other non-muscle cell sources (202,203). Therefore, SkM-EV translation should be viewed as an emerging and largely preclinical field, whereas broader EV-based therapy provides useful but non-equivalent experience in manufacturing, dosing, safety monitoring and regulatory evaluation.

Representative clinical studies and systematic analyses demonstrate the translational landscape of EV-based therapy (204,205). In degenerative musculoskeletal disease, intra-articular administration of allogeneic small EVs derived from UC-MSCs is being evaluated for knee osteoarthritis in dose-escalation or early safety-oriented studies (206,207). In inflammatory and regenerative indications, BMSC-derived EVs, including ExoFlo (Direct Biologics), have been evaluated in a Phase II double-blinded, placebo-controlled randomized trial for coronavirus disease 2019 (COVID-19)-associated moderate-to-severe acute respiratory distress syndrome (204).

Other studies are assessing nebulized MSC-derived EVs for COVID-19-associated lung injury, including aerosol inhalation of human adipose-derived MSC exosomes and nebulization therapy with UC-MSC-derived exosomes in patients with COVID-19-associated pneumonia (208,209). These studies indicate that EV therapeutics are entering early clinical development across several indications, but most clinical products are not SkM-derived.

For SkM-EV-based intervention, translational gaps must be addressed before clinical testing. These include defining a reproducible donor cell or tissue source, establishing scalable and GMP-compatible production, developing potency assays associated with disease-relevant mechanisms, verifying target tissue biodistribution and biologically meaningful tissue exposure, determining safe and biologically active dosing schedules, and assessing whether exercise-associated or engineered SkM-EV cargo molecules produce reproducible therapeutic effects in humans. Clinical experience from MSC-EVs and other EV products provides useful reference points, but cannot be directly extrapolated to SkM-EV-based interventions without source-specific characterization, human validation, disease-relevant potency assessment and clear regulatory classification.

7. Discussion

Despite evidence from SkM-EV profiling, muscle remodeling studies and muscle-organ crosstalk research showing SkM-EVs participate in local SkM remodeling and interorgan communication, controversies, methodological weaknesses and translational barriers remain unresolved, particularly regarding EV nomenclature, isolation strategies, tissue-origin attribution, biodistribution, cargo-function causality and clinical-grade standardization (3,7,210).

Contradictory findings and controversies. A persistent controversy concerns the biological identity of vesicle populations described as 'exosomes'. Many studies classify isolated vesicles as exosomes on the basis of particle size, morphology or tetraspanin enrichment, without direct evidence of endosomal biogenesis (3,211). Therefore, many SkM studies are more accurately described as studies of SkM-EVs, small EVs or exosome-like vesicles rather than biogenesis-defined exosomes (212,213). This distinction is important because vesicle subtypes may differ in cargo sorting, release mechanisms, biodistribution and recipient cell effects.

Another issue is the systemic contribution of SkM-EVs. Although SkM releases vesicles with distinct protein and miR signatures, evidence from sedentary mice and mice immediately following acute exhaustive treadmill running indicates that SkM-EVs may accumulate primarily within the SkM interstitium and contribute a limited fraction of circulating plasma EVs (35). Thus, myomiRs or muscle-enriched proteins detected in plasma EV preparations should not be interpreted as definitive evidence of SkM origin unless supported by tissue-specific labeling, genetic tracing, validated SkM-EV markers or other orthogonal source validation approaches (214). This is especially relevant for exercise-induced circulating EV profiles because exercise can simultaneously mobilize EVs from platelets, endothelial and immune cells, adipose tissue and other organs (186,187).

Contradictory findings are also evident in exercise- and aging-associated EV profiles. Exercise has been reported to alter circulating EV abundance, EV-associated protein markers and myomiR cargo profiles, but the magnitude and direction of these changes vary across studies (146,148-151,186,187). Similarly, aging-related changes in miR-1, miR-133a, miR-133b and miR-206 are inconsistent between human, animal and cell-based studies (31,215). These discrepancies may reflect differences in species, participant characteristics, muscle type, exercise protocol, sampling time, EV isolation method, normalization strategy and analytical platform. Therefore, SkM-EV cargo molecules should be interpreted as context-dependent molecular signals rather than fixed biomarkers of SkM status.

Methodological limitations of current evidence. Methodological heterogeneity remains a notable limitation in SkM-EV research. EV isolation methods, including differential ultracentrifugation, precipitation-based approaches, size-exclusion chromatography, ultrafiltration, density-gradient separation and immunoaffinity capture, differ substantially in yield, apparent purity, scalability and cargo recovery (216,217). As a result, EV preparations contain variable amounts of lipoproteins, protein aggregates, ribonucleoprotein complexes or other non-vesicular extracellular particles, which influence proteomic, miR and functional readouts (218). This issue is key when biological activity is attributed to a specific EV cargo molecule, because co-isolated non-vesicular components may contribute to the observed phenotype.

EV dose normalization and functional validation are inconsistent across studies (219,220). EV dose has been reported according to particle number, protein concentration, donor cell number, tissue weight or biofluid volume, limiting direct comparison between studies (219). In addition, numerous reports demonstrate associations between EV cargo changes and recipient-cell phenotypes, whereas fewer studies use rigorous loss-of-function, cargo-depletion, rescue or tissue-specific EV tracing approaches (220-222). Future studies should combine standardized EV characterization, quantitative biodistribution, orthogonal labeling strategies and functional rescue experiments to establish causal associations between SkM-EV cargo molecules and disease-relevant outcomes.

The heterogeneity of evidence levels limits interpretation. Many mechanistic findings are derived from C2C12 myoblasts, differentiated myotubes or primary SkM cells (35,43). Animal studies provide *in vivo* support but are typically based on rodent injury, atrophy, osteoporosis, ischemia or renal fibrosis models, which do not fully reproduce human aging, multimorbidity or chronic degenerative disease progression (64,79). Human evidence remains limited and is typically based on circulating EV profiling, myomiR measurements or observational associations following exercise or disease exposure (152,159,223). Therefore, conclusions derived from cell-based or animal studies should be labeled as *in vitro* or preclinical evidence and should not be directly extrapolated to clinical efficacy in humans.

Barriers to clinical translation and future directions. The clinical translation of SkM-EV-based interventions remains challenging because therapeutic preparations require

standardized donor cell sources, reproducible culture conditions, scalable production and consistent cargo profiles. Target-tissue delivery and enrichment also remain inefficient and context-dependent, particularly for organs such as bone, brain, heart and kidney (196,199). In addition, clinical-grade EV products require validated potency assays, batch-to-batch reproducibility, sterility testing, long-term stability, safety evaluation and predefined product-release criteria that must be met before clinical use, such as purity, sterility, endotoxin levels, cargo stability and potency (224). Regulatory classification remains complex because engineered EVs may share features of biological agents, cell-derived products, drug-delivery systems and gene therapy-like platforms depending on their source, modification strategy and therapeutic cargo molecules (195,224).

Future SkM-EV translation should prioritize whether defined SkM-EV subpopulations can be reproducibly produced, whether their tissue origin and biodistribution can be verified, whether specific cargo molecules are necessary and sufficient for therapeutic effects, whether repeated administration is safe and whether engineered SkM-EVs can meet clinically compatible release criteria. Addressing these issues requires source-specific quality control, quantitative biodistribution analysis, standardized potency assays and clinically relevant disease models before SkM-EV-based interventions can advance from experimental systems to early human therapeutic testing.

Limitations. The present review has several limitations. As a narrative review, it does not provide a quantitative synthesis of effect sizes, study quality or publication bias. Interpretation of the literature is further limited by heterogeneity in EV nomenclature, isolation methods, characterization depth, donor cell sources, experimental models, exercise protocols, disease context and outcome measurements. Although MISEV2023-consistent terminology was adopted, numerous studies do not provide sufficient evidence to distinguish biogenesis-defined exosomes from other small EVs or non-vesicular particles (3,213). Moreover, most mechanistic evidence remains preclinical, with limited direct human validation of SkM-EV-mediated interorgan communication (184,225). Because the present review focuses on SkM-EVs in multi-organ degenerative disease, it does not comprehensively cover all EV-producing tissues, disease categories or engineered EV platforms. These limitations underscore the need for standardized methodology, tissue-specific validation and clinically relevant models in future SkM-EV research.

8. Conclusion

SkM-EVs represent candidate vesicular mediators linking SkM with local and distant tissue through multimodal cargo molecules, including proteins, regulatory RNAs, lipids and metabolites. SkM-EVs may participate in SkM remodeling, neuromuscular communication, muscle-bone crosstalk, cardiac adaptation, renal fibrosis modulation and immune regulation but their biological effects are highly context-dependent and are shaped by donor muscle status, cargo composition, recipient cell identity, disease stage and experimental model. Under physiological or regenerative conditions, SkM-EVs support myogenic differentiation, satellite cell activation, extracellular matrix remodeling and tissue repair, whereas disease-conditioned SkM-EVs transmit inflammatory, catabolic, senescence-associated or anti-myogenic signals. Exercise, aging, alcohol exposure and metabolic stress can remodel SkM-EV release and cargo profiles, while exogenous plant-derived vesicle-like particles and irisin-related signaling may influence SkM remodeling or intersect with SkM-EV biology, although their direct effects on endogenous SkM-EV release and cargo sorting require validation. Despite progress, most mechanistic and therapeutic evidence remains preclinical, and direct human evidence validating SkM-EV-mediated interorgan communication or therapeutic efficacy is limited. Future studies should move beyond descriptive cargo profiling and integrate standardized EV isolation and characterization, tissue-specific source tracing, loss- and gain-of-function validation, quantitative biodistribution analysis, mechanism-based potency assays, clinically relevant disease models, GMP-compatible manufacturing and well-designed clinical studies. These advances are key for determining whether SkM-EV-based or SkM-EV-inspired strategies can be developed into reliable targeted interventions for metabolic and degenerative disease.

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Authors' contributions

XW, CG, WZ and WL wrote the manuscript. XW and GW constructed figures. LZ, BiG and BoG revised the manuscript. Data authentication is not applicable. All authors have read approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, the authors used artificial intelligence-assisted tools (including ChatGPT) to improve the readability and language of the manuscript. No artificial intelligence tool was used to generate scientific content, analyse or interpret data, draw scientific conclusions or create figures. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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