Research progress on the effects of macrophage-derived exosomes on muscle factors IGF-1 and FGF-2 mediating musculoskeletal crosstalk molecular signaling pathway on bone metabolism (Review)

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Abstract. Musculoskeletal crosstalk is essential for maintaining the balance of bone metabolism, with macrophage-derived exosomes emerging as key regulators of this process. Exosomes, small extracellular vesicles secreted by cells, carry a variety of bioactive molecules; proteins, lipids, mRNAs and miRNAs and facilitate intercellular communication by transferring these cargos to recipient cells. Specifically, macrophage-derived exosomes mediate muscle-bone interactions by transferring key regulators such as insulin-like growth factor-1 (IGF-1) and fibroblast growth factor-2 (FGF-2), thereby playing a pivotal role in bone metabolic homeostasis. Macrophages are classified into pro-inflammatory M1 and anti-inflammatory M2 phenotypes, each performing distinct functions in immune responses. Exosomes from M1 macrophages typically carry pro-inflammatory factors that can activate osteoclastic bone resorption, disrupting bone metabolism in pathological conditions. By contrast, exosomes from M2 macrophages often contain anti-inflammatory factors that promote tissue repair and bone formation. In the context of bone metabolism, exosomes from M1 and M2 macrophages modulate muscle-bone signaling by delivering regulators that influence the expression of IGF-1 and FGF-2, affecting osteoblast proliferation, differentiation, and mineralization. M1 macrophage-derived exosomes activate signaling pathways such as NF-κB and MAPK through the transfer of pro-inflammatory cargo, thereby enhancing bone resorption. By contrast, exosomes from M2 macrophages can suppress pro-inflammatory signaling while activating pathways like TGF-β and PI3K/Akt, promoting bone synthesis and repair. As critical myokines, IGF-1 and FGF-2 not only support muscle growth, repair, and maintenance but also directly influence bone remodeling through musculoskeletal crosstalk.

Contents

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1. Introduction

Musculoskeletal crosstalk is a fundamental physiological process that maintains human health, involving the dynamic interaction and regulation between bone and muscle through intricate molecular signaling mechanisms (1). Myokines such as insulin-like growth factor-1 (IGF-1) and fibroblast growth factor-2 (FGF-2) are pivotal in mediating this crosstalk (2). IGF-1 regulates muscle growth and metabolism by promoting myocyte proliferation and repair, while FGF-2 plays a critical role in muscle regeneration and repair (3). Notably, both IGF-1 and FGF-2 not only regulate muscle growth but also markedly influence bone metabolism, bone density maintenance and the repair and regeneration of bone tissue (4). This inter-system regulatory mechanism highlights the importance of musculoskeletal crosstalk as a critical area of research (5). Recent developments in intercellular communication have identified macrophage-derived exosomes as emerging signaling mediators, gaining increasing attention in the scientific community (6). Exosomes are small extracellular vesicles secreted by cells that carry a range of bioactive molecules, including proteins, RNAs, and lipids, facilitating intercellular communication and the regulation of cellular functions and tissue physiology (7).

Macrophages regulate musculoskeletal interactions through the secretion of exosomes (8). Exosomes derived from M1 and M2 macrophages exhibit distinct effects on musculoskeletal metabolism (Tables I and II) (9-13). M1 macrophages, which are pro-inflammatory, release exosomes enriched with pro-inflammatory cytokines that can stimulate osteoclastic bone resorption, potentially contributing to osteoporosis and bone damage. By contrast, M2 macrophages, which possess anti-inflammatory properties, secrete exosomes containing anti-inflammatory factors that promote bone formation and repair. Macrophages modulate muscle-bone signaling through the secretion of specific exosome subtypes. Exosome-associated factors like IGF-1 and FGF-2 regulate bone metabolic homeostasis, either promoting or inhibiting bone remodeling.

The present review aimed to synthesize recent insights into macrophage-derived exosomes and their role in regulating musculoskeletal crosstalk, with a focus on their involvement in IGF-1- and FGF-2-mediated signaling pathways. It examined how exosomes influence the functions of these key factors in bone metabolism, elucidated their roles in muscle and bone homeostasis and suggested future research avenues. Additionally, it sought to provide a theoretical framework for the development of therapeutic strategies targeting exosomes, particularly for the treatment of bone metabolic disorders and musculoskeletal diseases (14).

2. The importance of muscle factors IGF-1 and FGF-2 in musculoskeletal crosstalk

Within the musculoskeletal system, muscle-derived factors such as IGF-1 and FGF-2 are essential in mediating tissue crosstalk (Table III) (15-18). These factors coordinate the development and repair of both bone and muscle by regulating their bidirectional interactions. IGF-1 is a pivotal growth factor that promotes muscle cell proliferation and differentiation while also exerting significant regulatory effects on the skeletal system (19). Binding to its receptor activates the PI3K/Akt pathway, fostering muscle growth and enhancing bone mineralization and density, thereby serving as a critical link between bone and muscle (20). FGF-2 is a key regulator of fibroblasts and bone marrow-derived cells, playing an indispensable role in bone repair and muscle regeneration (21). Upon binding to fibroblast growth factor receptors (FGFRs), FGF-2 activates downstream pathways, including MAPK/ERK and PI3K/Akt, that promote the growth and differentiation of osteoblasts and myocytes (22). Notably, FGF-2 supports effective repair of muscle and bone tissues following exercise or trauma.

3. Musculoskeletal crosstalk molecular signaling pathways

The physiological connection between muscle and bone

The role of mechanical signal transduction in musculoskeletal interaction. Mechanical signal transduction is crucial in musculoskeletal interactions (23). The close relationship between bone and muscle during exercise is particularly dependent on mechanical stimulation (Fig. 1) (23,24). During exercise, muscles generate mechanical tension on bones, stimulating bone growth and remodeling, while also modulating various aspects of bone metabolism (25). For instance, during weight-bearing exercise, muscle contraction generates mechanical loads, such as pressure and traction, on bone. These forces are transmitted via mechanoreceptors on bone cells, regulating the balance between bone resorption and formation (26). In response to mechanical stimuli, bone activates several signaling pathways, including mechanosensory pathways such as the Yes-associated protein/transcriptional coactivator with PDZ-binding motif (YAP/TAZ) pathway, which activates osteoblasts and other bone-related cells to promote bone mineralization (27). Mechanical signals also regulate muscle growth (28). Muscle adapts to mechanical loads through signaling molecules such as growth factors and protein kinases, which promote muscle fiber growth and proliferation (29). This feedback mechanism fosters muscle hyperplasia while simultaneously influencing bone, facilitating integrated musculoskeletal crosstalk and highlighting the interdependence of bone and muscle throughout human development (30).

The role of endocrine factors in musculoskeletal communication. Beyond mechanical signals, endocrine factors also play a vital role in musculoskeletal crosstalk (31). Muscles and bones mutually regulate and support each other via various endocrine factors, including IGF-1, growth hormone, sex hormones and FGF-2 (Fig. 2) (32). During growth, IGF-1 secreted by muscles promotes muscle development and, via circulation, also affects the skeletal system, facilitating bone formation (33). Binding to the IGF-1 receptor (IGF-1R) in bone activates the PI3K-Akt and MAPK pathways, regulating osteoblast proliferation and differentiation (34). Sex hormones, particularly estrogen, are critical in regulating muscle-bone interactions (35). Estrogen maintains bone density and mass by promoting bone formation and inhibiting resorption, while also enhancing musculoskeletal synergy by regulating muscle strength and endurance (Fig. 3) (35). FGF-2, as an endocrine factor in bone, contributes to bone repair and regulates growth and mineralization by binding to FGFRs (Fig. 2) (36).

Table I. Comparative analysis of M1 vs. M2 macrophage-derived exosomes in bone metabolism regulation.

Parameter	M1-Exos	M2-Exos
Biogenesis	From LPS/IFN-γ-polarized macrophages	Produced by IL-4/IL-13-stimulated macrophages
Surface Markers	$CD86^+$, $iNOS^+$, $MHC\text{-}II^+$	$CD206^+$, $Arg\text{-}1^+$, $CD163^+$
Key cargos	miRNAs: miR-155, let-7c Proteins: TNF-α, IL-6, IL-1β	miRNAs: miR-223, miR-146a Proteins: IL-10, TGF-β, CCL18
Osteoblast effects	Inhibits differentiation via Wnt/β-catenin suppression ALP activity↓	Promotes mineralization via BMP/Runx2 pathway COL1A1 and OCN expression ↑
Osteoclast effects	Enhances RANKL-induced differentiation $TRAP^+$ multinucleated cells↑	Secretes OPG to inhibit RANKL Resorption pit area ↓ (by 40-60%)
Metabolic reprogramming	Glycolysis↑ (HK2/LDHA↑) ROS production↑	Oxidative phosphorylation↑ (ATP5A1↑) FAO via PPARγ activation
Therapeutic potential	Acute bone injury (such as fracture hematoma clearance)	Chronic conditions (such as osteoporosis, non-union fractures)
(Refs.)	(9,10)	(11)

Upward arrow (↑) indicates increase; downward arrow (↓) indicates decrease. M1-Exos, M1 macrophage-derived exosomes; M2-Exos, M2 macrophage-derived exosomes; LPS, lipopolysaccharide; IFN-γ, interferon γ; $iNOS^+$, inducible nitric oxide synthase; MHC-II, major histocompatibility complex class II; miRNAs/miRs, microRNAs; ALP, alkaline phosphatase; OPG, osteoprotegerin; TGF-β, transforming growth factor β; ROS, reactive oxygen species; BMP, bone morphogenetic protein; OCN, osteocalcin; RANKL, receptor activator of nuclear factor-κB ligand; HK2, hexokinase 2; LDHA, lactate dehydrogenase A; ATP5A1, ATP synthase-alpha; PPARγ, peroxisome proliferator-activated receptor gamma; FAO, fatty acid oxidation; RUNX2, Runt-related transcription factor 2.

Table II. Comparative analysis of M1 and M2 macrophage-derived exosomes in muscle metabolism regulation.

Parameter	M1-Exos	M2-Exos
Polarization signal	Induced by LPS/IFN-γ	Induced by IL-4/IL-13
Key cargos	miRNAs: miR-155, miR-27a Proteins: TNF-α, IL-6, IL-1β	miRNAs: miR-223, miR-206 Proteins: IL-10, IGF-1, TGF-β
Myogenesis	Inhibits satellite cell differentiation (MyoD↓) Promotes atrophy (MuRF-1↑)	Enhances myoblast fusion (MyoG↑) Reduces fibrosis (Collagen III↓)
Glucose metabolism	Induces insulin resistance (IRS-1 phosphorylation↓) GLUT4 translocation↓	Improves insulin sensitivity (Akt activation↑) Glycogen synthesis↑
Lipid metabolism	Promotes lipolysis (ATGL↑) Mitochondrial dysfunction (ROS↑)	Enhances fatty acid oxidation (CPT1B↑) Mitochondrial biogenesis (PGC-1α↑)
Clinical relevance	Chronic inflammation (such as sarcopenia, DMD)	Muscle regeneration (such as injury, aging)
(Refs.)	(12)	(13)

Upward arrow (↑) indicates increase; downward arrow (↓) indicates decrease. M1-Exos, M1 macrophage-derived exosomes; M2-Exos, M2 macrophage-derived exosomes; LPS, lipopolysaccharide; IGF1, insulin-like growth factor 1; TGF-β, transforming growth factor beta; MyoD, myogenic differentiation 1; MuRF1, muscle ring finger-1; MYOG, human myogenin; IRS1, insulin receptor substrate 1; Akt, protein kinase B; GLUT4, Glucose transporter type-4; ROS, reactive oxygen species; CPT1B, Carnitine palmitoyltransferase 1b; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator 1α; DMD, Duchenne muscular dystrophy.

Particularly during aging or following fracture, FGF-2 supports bone repair by stimulating osteoblast proliferation and differentiation (37).

Molecular signaling pathways in musculoskeletal crosstalk mediated by IGF-1 and FGF-2

Mechanisms of IGF-1-mediated signaling pathways in musculoskeletal crosstalk. As a pivotal growth factor in muscle-bone communication, IGF-1 regulates musculoskeletal crosstalk through multiple signaling pathways (Fig. 4) (38). Upon binding to its receptor, IGF-1 activates the PI3K/Akt pathway, promoting muscle fiber proliferation and repair while modulating bone cell metabolism (39). In the skeletal system, the PI3K/Akt pathway influences osteoblast and osteoclast activity via downstream mTOR signaling, thus regulating bone formation and resorption (40). Activation of PI3K/Akt leads to Akt kinase

Table III. Comparative roles of IGF-1 and FGF-2 in muscle and bone metabolism.

Category	IGF-1	FGF-2
Primary source	Liver (endocrine), muscle/osteoblasts (paracrine)	Mesenchymal stem cells, osteoblasts, damaged tissues
Muscle metabolism	Anabolic Effects: Activates PI3K/Akt/mTOR → protein synthesis↑ Suppresses FoxO/MuRF-1 → atrophy↓ Enhances satellite cell differentiation	Proliferative Effects: Binds FGFR1→MAPK → myoblast proliferation↑ Synergizes with IGF-1 for regeneration Reduces fibrosis (TGF-β inhibition)
Bone metabolism	Pro-osteogenic: Stimulates Runx2/Osterix → mineralization↑ Inhibits RANKL → osteoclastogenesis↓ ECM production (Collagen I↑)	Biphasic Action: Low dose: BMP-2 synergy → osteogenesis↑ High dose: Angiogenesis (VEGF/FGFR1)→ bone remodeling
Metabolic influence	GLUT4 translocation↑ → glucose uptake↑ PPARδ activation → fatty acid oxidation↑	Glycolysis↑ via HIF-1α Modulates bone-vascular coupling (PDGF-BB interaction)
Receptor pathway	IGF-1R→IRS-1→PI3K/Akt	FGFR1-4→RAS/MAPK or PLCγ
Therapeutic use	rhIGF-1 for sarcopenia Bone defect scaffolds (sustained release)	FGF-2-coated implants Muscle injury repair (clinical trials)
(Refs.)	(15,16)	(17,18)

Upward arrow (↑) indicates increase; downward arrow (↓) indicates decrease. IGF-1, Insulin-like growth factor-1; FGF-2, Fibroblast growth factor-2; PI3K, phosphatidylinositol-3-kinase; Akt, protein kinase B; mTOR, mammalian target of rapamycin; FoxO, Forkhead box O; MuRF-1, muscle RING finger-1; FGFR, Fibroblast growth factor receptor; MAPK, Mitogen-activated protein kinase; TGF-β, Transforming growth factor beta; Runx2, Runt-related transcription factor 2; RANKL, Receptor activator of nuclear factor kappa-B ligand; ECM, Extracellular matrix; BMP-2, Bone morphogenetic protein-2; VEGF, Vascular endothelial growth factor; GLUT4, glucose transporter type 4; PPARδ, Peroxisome proliferator-activated receptor delta; HIF-1α, hypoxia-inducible factor 1-alpha; PDGF-BB, Platelet-derived growth factor BB; RAS, rat sarcoma; PLCγ, phospholipase C gamma; rhIGF-1, recombinant human IGF-1.

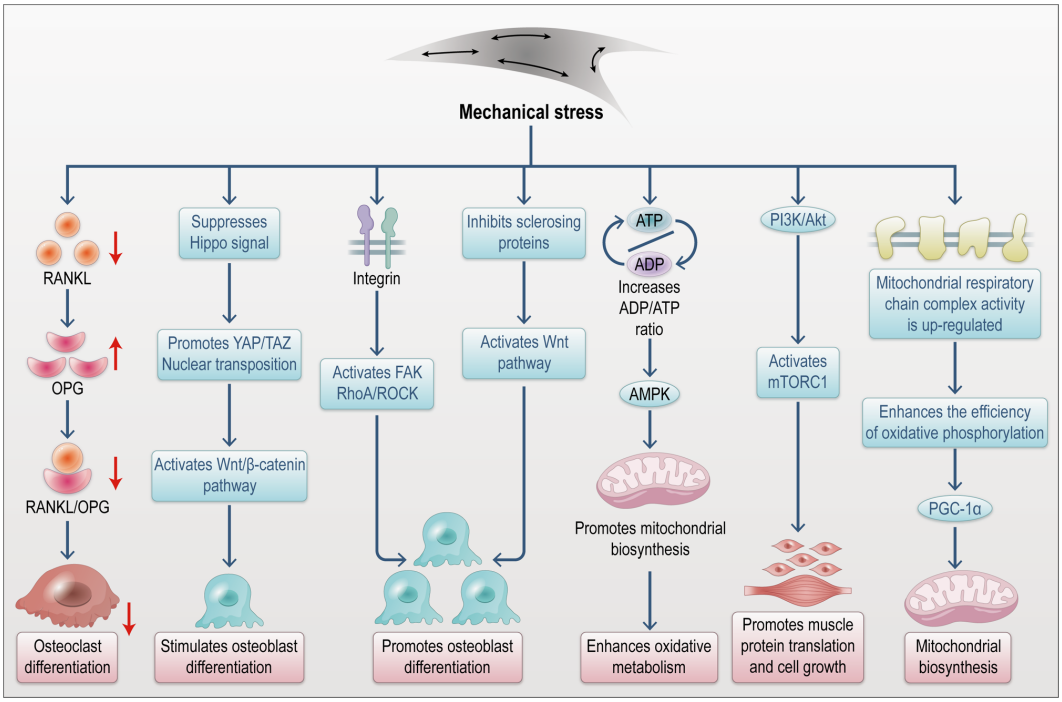

Figure 1. Regulatory mechanism of mechanical signal transduction on the musculoskeletal system. Mechanical stress inhibits the Hippo pathway, activating Wnt/β-catenin signaling. It also suppresses sclerostin, further promoting Wnt pathway activation. Additionally, mechanical stress regulates integrins to activate the FAK and RhoA/ROCK pathways, stimulating osteoblasts and other bone-related cells, thus promoting bone mineralization. Furthermore, mechanical stress regulates muscle growth through activation of AMPK, PI3K/Akt/mTORC1, and mitochondrial metabolism-related pathways. FAK, Focal adhesion kinase; RhoA, Ras homolog gene family member A; ROCK, Rho-associated kinase; AMPK, Adenosine monophosphate-activated protein kinase; PI3K, phosphatidylinositol-3-kinase; Akt, protein kinase B; mTORC1, mammalian target of rapamycin complex 1; PGC-1α, Peroxisome proliferators-activated receptor γ coactivator 1α.

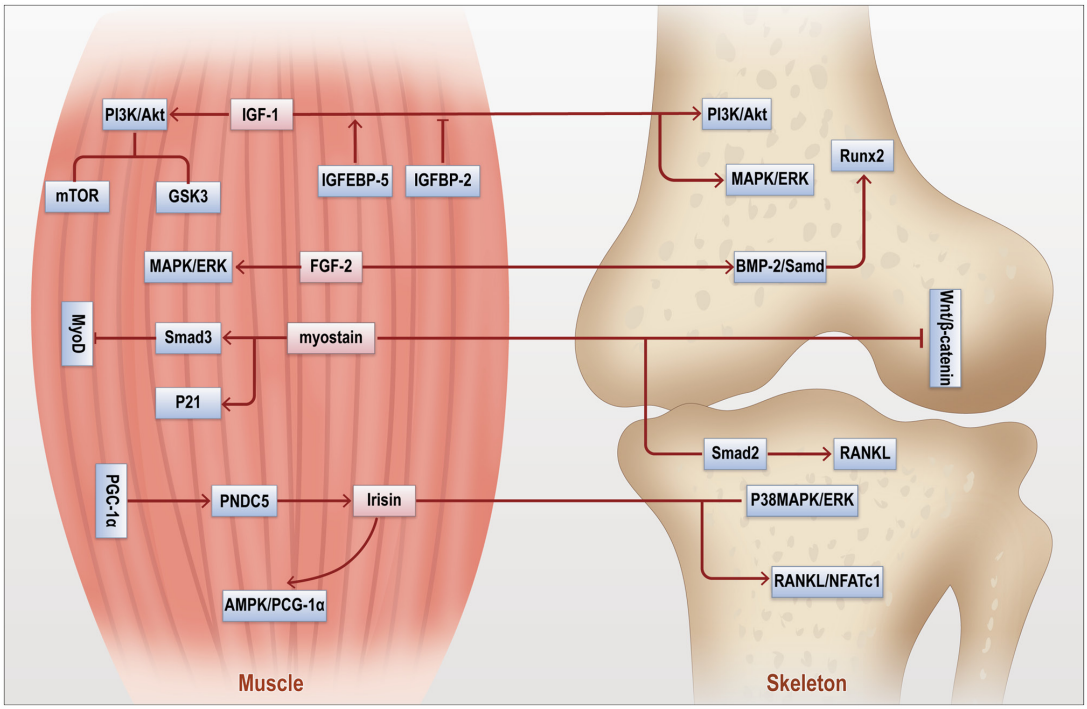

Figure 2. Regulatory pathways of muscle factors on the musculoskeletal system. IGF-1 modulates muscle metabolism via the PI3K/Akt/mTOR pathway and bone metabolism through PI3K/Akt and MAPK/ERK signaling. FGF-2 regulates muscle metabolism via MAPK/ERK and bone metabolism through BMP-2/Smad signaling. Myostatin influences muscle metabolism via Smad3 and P21, and bone metabolism through Smad2 and Wnt/β-catenin pathways. PGC-1α stimulates PNDC5, promoting Irisin release from muscle; Irisin subsequently regulates bone metabolism via P38MAPK/ERK and RANKL/NFATc1 signaling. IGF-1, Insulin-like growth factor-1; FGF-2, Fibroblast growth factor 2; GSK3, Glycogen Synthase Kinase-3; IGFBP-3, insulin-like growth factor binding protein 3; IGFBP-5, insulin-like growth factor binding protein 5; MyoD, Myoblast determination protein 1; PNDC, peroxynitrite decomposition catalyst; Irisin, human recombinant; Runx2, Runt-related transcription factor 2; PI3K, phosphatidylinositol-3-kinase; Akt, protein kinase B; mTOR, mammalian target of rapamycin; PGC-1α, peroxisome proliferators-activated receptor γ coactivator 1α; RANKL, receptor activator of nuclear factor-κB ligand; NFATc1, nuclear factor of activated T cells; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; BMP-2, bone morphogenetic protein type 2; Smad3, mothers against decapentaplegic homolog 3; Wnt, wingless-related integration site.

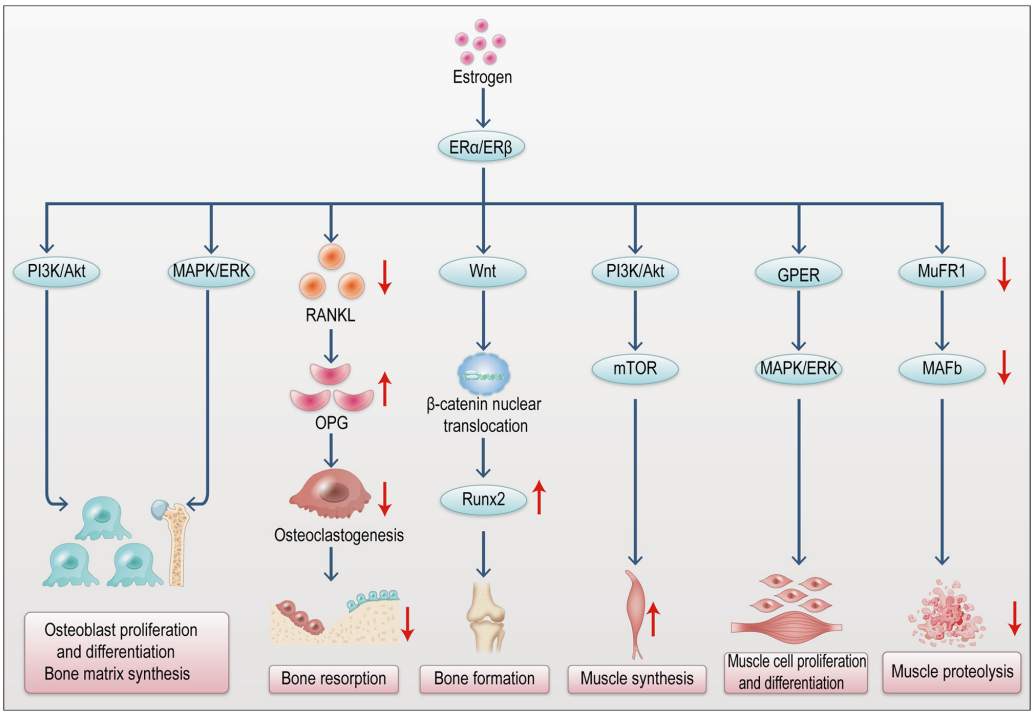

Figure 3. Regulatory mechanism of estrogen on the musculoskeletal system. Estrogen modulates bone metabolism through PI3K/Akt, MAPK/ERK, RANKL/OPG, and Wnt/β-catenin pathways. In muscle, estrogen influences metabolism via PI3K/Akt/mTOR, GPER/MAPK/ERK, RANKL/OPG, and MuRF1/MAFbx pathways. GPER, G protein-coupled estrogen receptor; ERβ, estrogen receptor beta; ERα, estrogen receptor alpha; Runx2, Runt-related transcription factor 2; PI3K, phosphatidylinositol-3-kinase; Akt, protein kinase B; mTOR, mammalian target of rapamycin; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; RANKL, receptor activator of nuclear factor-κB ligand; OPG, osteoprotegerin; MAFbx, muscle atrophy F-box; MuRF1, muscle RING finger-1.

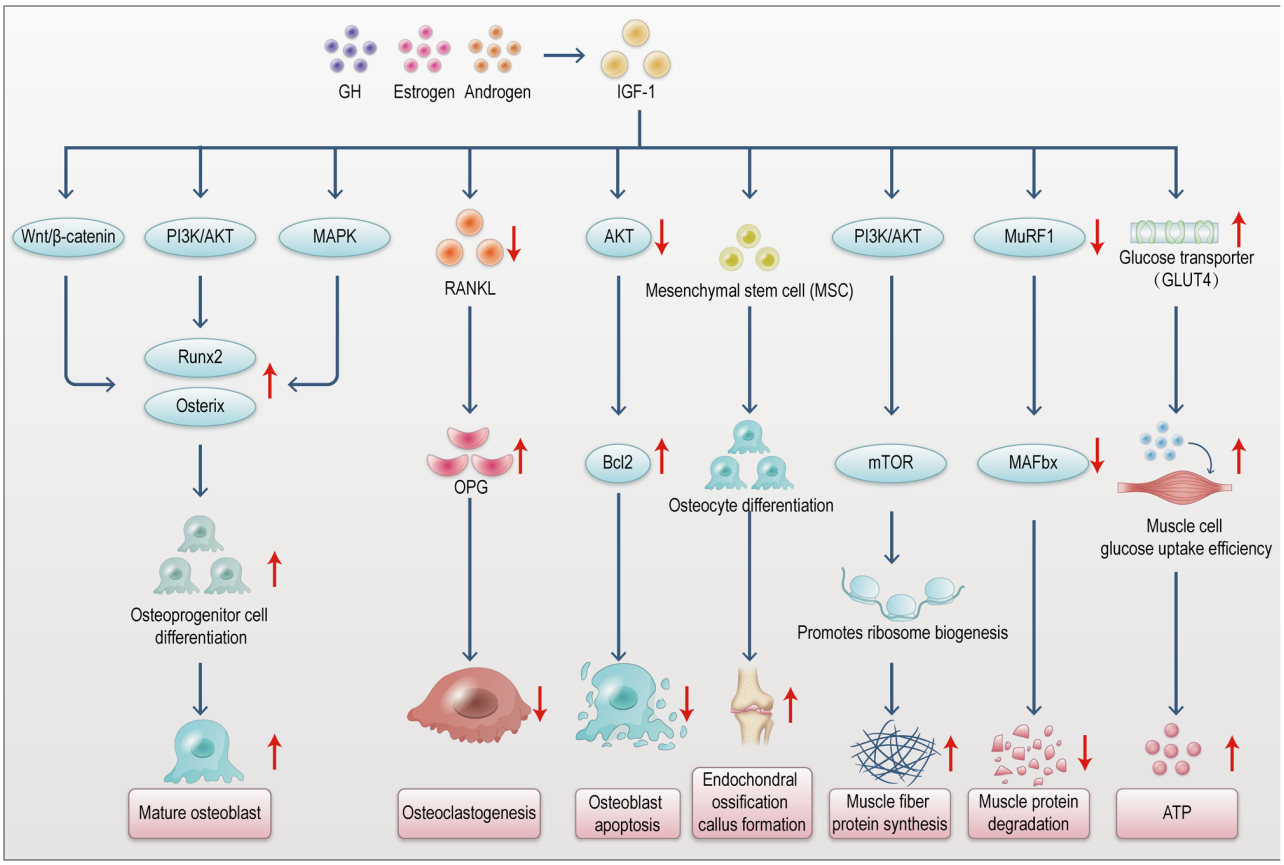

Figure 4. Regulatory mechanism of IGF-1 on the musculoskeletal system. IGF-1 modulates bone metabolism through several signaling pathways, including Wnt/β-catenin, PI3K/Akt, MAPK, RANKL/OPG, and Akt. It also influences muscle metabolism via the PI3K/Akt/mTOR, MuRF1/MAFbx, and GLUT4 pathways. Wnt, ςingless-type MMTV integration site family; Bcl-2, B-cell lymphoma-2; GH, growth hormone; IGF-1, insulin-like growth factor 1; PI3K, phosphatidylinositol-3-kinase; Akt, protein kinase B; mTOR, mammalian target of rapamycin; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; RANKL, receptor activator of nuclear factor-κB ligand; OPG, osteoprotegerin; MAFbx, muscle atrophy F-box; MuRF1, muscle RING finger-1; GLUT4, Glucose transporter protein 4.

phosphorylation, which in turn activates proteins involved in cell growth and metabolism, promoting proliferation, survival and differentiation (41). In bone, the PI3K/Akt pathway is essential for bone formation by stimulating osteoblast proliferation and differentiation, while enhancing bone matrix mineralization (42). IGF-1 activation of this pathway improves bone density and accelerates fracture healing, highlighting its role in bone repair (43).

The role of signal transduction pathways involved in FGF-2 [such as FGF receptor (FGFR)-related pathways] in musculoskeletal interactions. FGF-2 contributes to musculoskeletal crosstalk by promoting muscle growth and regulating bone formation and repair through the FGFR (Fig. 5) (44,45). In bone metabolism, FGF-2 modulates osteoblast and osteoclast functions, influencing bone formation and resorption via the MAPK/ERK signaling pathway (46). Research shows that FGF-2 enhances bone repair by stimulating osteoblast proliferation and migration, accelerating new bone formation during fracture healing (47). Additionally, FGF-2 helps maintain bone density by balancing osteoblast and osteoclast activity, thus reducing bone resorption (48). FGF-2 exerts a bidirectional regulatory effect in musculoskeletal crosstalk, promoting bone formation while inhibiting excessive bone resorption. In summary, both IGF-1 and FGF-2 are crucial in musculoskeletal interactions, regulating bone and muscle growth and repair through their respective signaling pathways and coordinating the development and repair of the musculoskeletal system through their interplay.

4. Macrophage-derived exosomes

Exosomes are small, bilayer lipid-enclosed vesicles secreted by cells, encompassing exosomes, microvesicles and apoptotic bodies (7). Among these, exosomes are the most extensively studied subtype (49). They carry a variety of cargo, including proteins, metabolites, nucleic acids and lipids, derived from their parent cells, exerting biological effects similar to those of the original cells (Table IV) (50-53). Exosomes can bind to recipient cells through surface ligands or be internalized via paracrine signaling and membrane fusion (such as endocytosis) (54), facilitating the transfer of bioactive molecules and modulating recipient cell functions. Macrophage-derived exosomes play critical roles in regulating inflammation and influencing the progression of bone diseases, such as osteoporosis, fractures and osteoarthritis, markedly affecting bone metabolism and homeostasis (55-57). Research indicates that both the composition and quantity of exosomes vary depending on the macrophage polarization state (58-60). In recent years, macrophage-derived exosomes have gained growing research interest, following extensive earlier studies on exosomes from mesenchymal stem cells (MSCs).

Table IV. Comprehensive composition and functions of macrophage-derived exosomal components.

Component category	Representative molecules	Biological functions
Proteins	• MHC class II (HLA-DR)	• Antigen presentation
	• Integrins (αMβ2)	• Tissue-specific homing
	• Heat shock proteins (HSP70/90)	• Protein folding and stress response
Nucleic acids	• miRNAs (such as miR-155, miR-21)	• Post-transcriptional gene regulation
	• lncRNAs (such as H19)	• Epigenetic modulation
	• mtDNA	• cGAS-STING pathway activation
Lipids	• Sphingomyelin (SM)	• Membrane stability and curvature
	• Cholesterol	• Inflammatory signaling modulation
	• Prostaglandin E2 (PGE2)	• Vascular permeability regulation
Metabolites	• Lactate	• Glycolytic metabolic signaling
	• Succinate	• Pro-inflammatory signal amplification
	• Glutathione (GSH)	• Redox homeostasis maintenance
Receptors/signaling	• TLR4	• Pathogen-associated molecular pattern recognition
	• TNF-α	• Pro-/anti-inflammatory signal transduction
	• TGF-β1	• Immunomodulation and tissue repair
(Refs.)	(50-53)	

MHC, major histocompatibility complex; HLA-DR, Human histocompatibility leukocyte antigen (HLA)-DR; cGAS, Cyclic GMP-AMP synthase; STING, Stimulator of interferon genes; TGF-β1, Transforming growth factor-beta 1; TNF-α, Tumor necrosis factor alpha; TLR4, Toll-like receptor 4; miRNAs, microRNAs; lncRNAs, long noncoding RNAs; mtDNA, Mitochondrial genome.

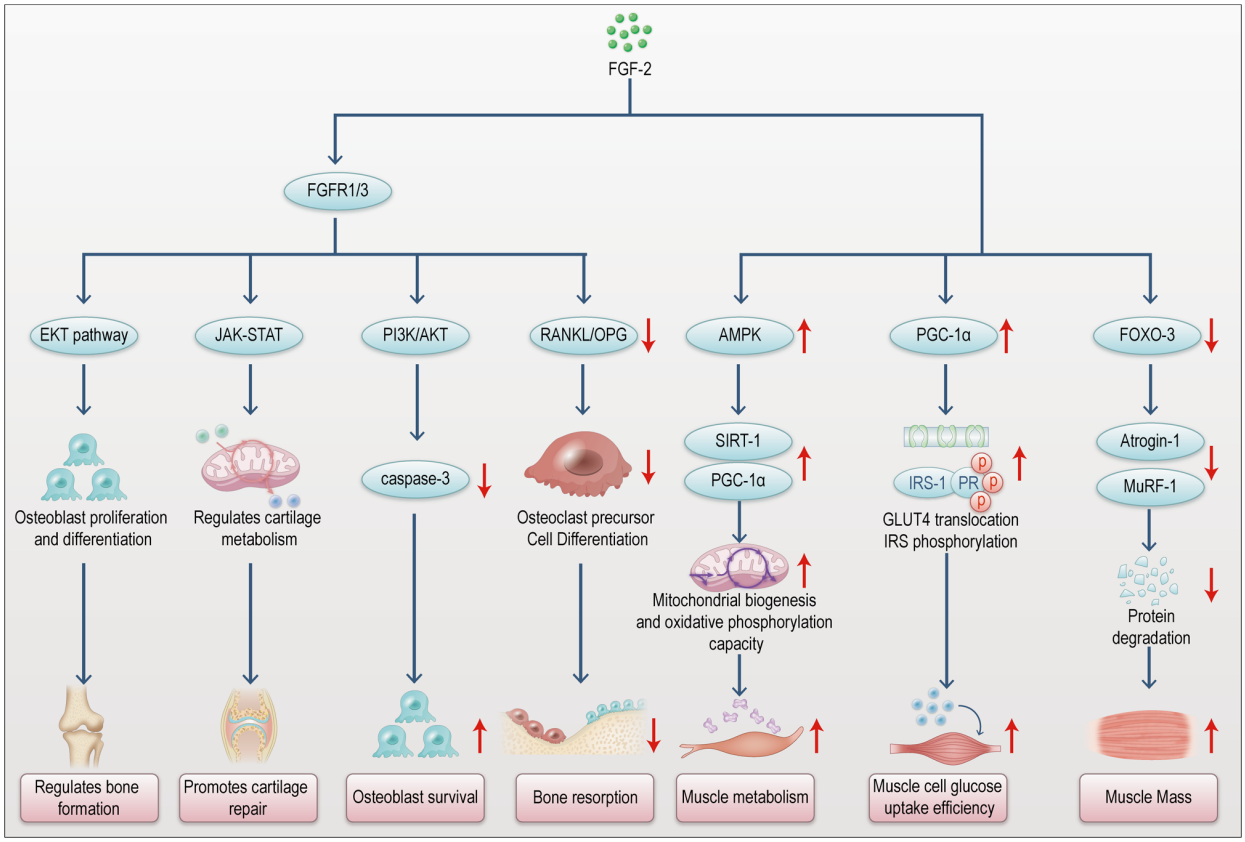

Figure 5. Regulatory mechanism of FGF-2 on the musculoskeletal system. FGF-2 regulates bone metabolism through the ERK, JAK-STAT, PI3K/Akt, and RANKL/OPG pathways. In muscle, it affects metabolism via AMPK/PGC-1α, AMPK/SIRT-1, PGC-1α/GLUT4, PGC-1α/IRS-1, FOXO-3/Atrogin-1 and FOXO-3/MuRF-1 signaling. FGF-2, Basic fibroblast growth factor; JAK, Janus kinase; STAT, Signal transducers and activators of transcription; PI3K, phosphatidylinositol-3-kinase; Akt, protein kinase B; mTOR, mammalian target of rapamycin; Atrogin-1, muscle atrophy-1; PR, progesterone receptors; FGFR, fibroblast growth factor receptor; AMPK, AMP-activated protein kinase; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1 alpha; MAPK, Mitogen-activated protein kinase; ERK, Extracellular signal-regulated kinase; RANKL, receptor activator of nuclear factor-κB ligand; OPG, Osteoprotegerin; MuRF1, muscle RING finger-1; GLUT4, Glucose transporter protein 4; SIRT1, Sirtuin1; IRS1, Insulin receptor substrate 1; FOXO-3, Forkhead box O3.

Macrophage-derived exosomes and their effect on musculoskeletal metabolism

The role of M1 macrophage-derived exosomes in bone metabolism. M1-polarized macrophages promote the release of chemotactic and inflammatory mediators, which facilitate osteoclast activation and debris clearance at fracture sites (61-63). MicroRNAs (miRNAs), highly conserved non-coding RNAs, post-transcriptionally regulate gene expression and play a key role in intercellular communication as integral components of exosomes (64). The miRNA profiles of M1 and M2 polarized macrophages exhibit significant differences. Kang *et al* (59) demonstrated that M1 exosomes are enriched with miR-155, which inhibits the bone morphogenetic protein (BMP) signaling pathway by downregulating BMP2, BMP9, and Runt-related transcription factor 2 (Runx2). Given that BMP2 is critical for osteoblast differentiation (65), its downregulation impedes bone regeneration. Ge *et al* (60) reported that exosomal miR-155 from M1 macrophages targets SOCS6, resulting in elevated p65 protein levels. This activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway exacerbates inflammation. Additionally, miR-222 is highly expressed in M1 macrophages (66). Studies have shown that exosomal miR-222 from M1 macrophages induces apoptosis in bone marrow mesenchymal stem cells (BMMSCs) by inhibiting Bcl-2 expression (67-69). Yu *et al* (70) demonstrated that exosomal miR-98 from M1 macrophages targets DUSP1 in MC3T3-E1 cells and a postmenopausal osteoporosis murine model, inhibiting osteogenic differentiation. Another study showed that M1 exosomes are enriched with miR-1246, which activates the Wnt pathway by downregulating GSK3β and Axin2, thereby promoting cartilage inflammation and degradation (71). These findings were primarily validated using bioinformatics analyses, sequencing and related methodologies. In summary, M1 macrophage-derived exosomes, which are enriched with miRNAs such as miR-155, miR-222, miR-98 and miR-1246, modulate downstream signaling pathways to promote inflammation and impair bone repair both *in vitro* and *in vivo*. The specific miRNA cargo determines the downstream signaling effects by targeting key nodes within signaling networks. Exosomes serve as specific carriers, enabling macrophage-derived miRNAs to function as promising biomarkers for monitoring and regulating bone remodeling. However, the complete repertoire of miRNAs and other cargo within macrophage exosomes remains incompletely characterized. Further investigation is needed to understand how macrophage polarization influences exosomal miRNA cargo and the precise mechanisms through which these miRNAs regulate downstream pathways. The effects of macrophage exosomes on BMMSC differentiation have been extensively studied. He *et al* (72) collected conditioned medium (CM) from M1 macrophages and observed enhanced BMMSC proliferation, adipogenic differentiation and extracellular matrix deposition. Upon isolating exosomes from this CM, it was found that M1 exosomes, but not M2 exosomes, promoted stem cell proliferation, osteogenesis and adipogenesis. This finding was corroborated by Xia *et al* (73). By contrast, Kang *et al* (59) reported that co-culture with M1 macrophages markedly reduced BMP2 and BMP9 expression in mouse MSCs, suggesting that M1 macrophage-derived exosomes impair the osteoinductive effects of BMPs and suppress osteogenic gene expression in MSCs. Through exosomal communication, M1 macrophages primarily enhance early and mid-stage osteogenesis, exerting stronger effects on BMMSC proliferation, osteogenesis and adipogenesis compared with M2 macrophages. These differing effects on osteogenesis may be attributed to variations in recipient cell sources. Experimental discrepancies could stem from multiple factors, including macrophage maturity, co-culture conditions, the presence of other cytokines in the CM, recipient cell lineage, and technical differences in exosome isolation from specific conditioned media (62,73). The balance between osteogenic and adipogenic differentiation in BMMSCs is essential for maintaining bone mass. Investigating the mechanisms by which M1 macrophage-derived exosomes regulate this balance in BMMSCs may offer insights into clinical disease pathogenesis and aid in the development of targeted therapies. Fig. 6 summarizes the regulation of bone remodeling signaling pathways by M1 macrophage-derived exosomes.

The role of M2 macrophage-derived exosomes in bone metabolism. A critical event in bone healing is the transition from the pro-inflammatory M1 macrophage phenotype to the anti-inflammatory M2 phenotype (74). In the later stages of bone healing, M2 macrophages establish an anti-inflammatory environment that promotes osteogenesis in BMMSCs. Li *et al* (56) identified exosomes as key mediators of the osteogenic process induced by M2 macrophages. BMPs are key endogenous regulators of osteogenesis, with the loss of BMP2 or BMP4 function leading to significant impairments in osteogenesis. BMPs activate Smad proteins and upregulate Runx2 expression, driving MSC differentiation into osteoblasts (75,76). Using an *in vivo* bone defect model, Kang *et al* (59) demonstrated that M2 macrophage-derived exosomes promote bone regeneration. Further analysis revealed that M2 exosomes are enriched with miR-378a, which enhances osteoinduction by modulating the BMP signaling pathway, thus promoting cranial bone regeneration in mice. Studies also indicate that exosomes from IL-4-stimulated M2 macrophages, enriched with miRNAs such as miR-99a-5p, miR-146b-5p and miR-378-3p, suppress inflammation by downregulating the TNF-α and NF-κB signaling pathways in $Apoe^{-/-}$ mice (77). Moreover, exosomes derived from M2 macrophages promote osteogenic differentiation of BMMSCs through miR-690, IRS-1 and TAZ, while inhibiting adipogenic differentiation (56). Yu *et al* (78) reported that miR-690, enriched in M2 macrophage-derived exosomes, upregulates osteogenic differentiation in C2C12 myogenic progenitor cells by inhibiting the translation of the NF-κB p65 protein. M2 macrophage-derived exosomes are also shown to carry high levels of miR-221-5p, which promotes tissue repair and regeneration by binding to the 3' untranslated region (UTR) of E2F2 mRNA and negatively regulating its expression in pancreatic ductal adenocarcinoma (79). Collectively, these studies suggest that M2 macrophage-derived exosomes infiltrate the bone marrow microenvironment and transfer specific miRNAs (such as miR-378a, miR-690, miR-99a-5p) to BMMSCs, facilitating osteogenic differentiation and fracture healing (80,81). These miRNAs likely function as pro-osteogenic agents by modulating key pathways such as BMP signaling to enhance osteogenesis. However, the precise roles of M2

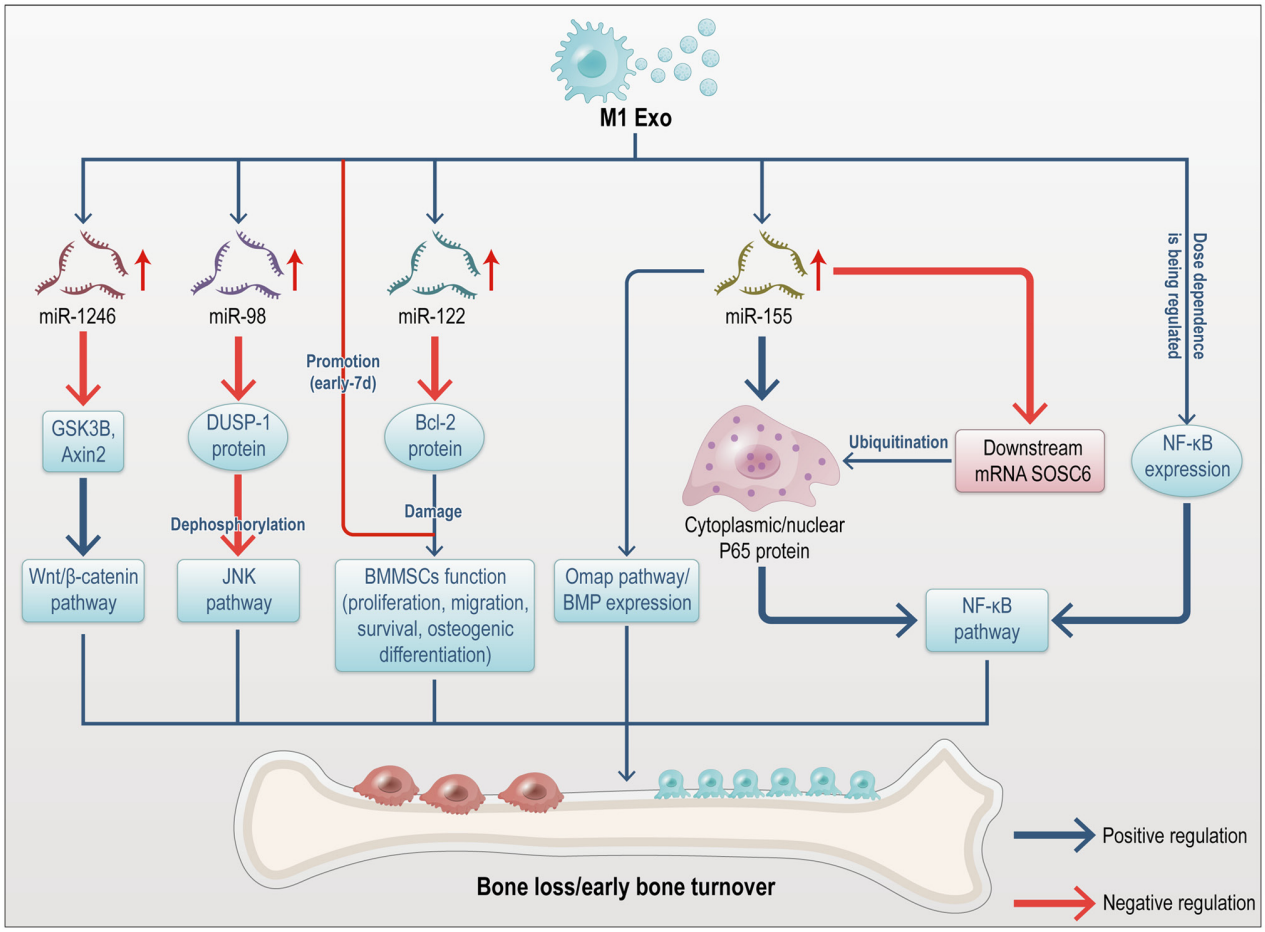

Figure 6. Regulation of bone remodeling signaling pathways by M1 exosomes. M1 macrophage-derived exosomes contain specific microRNAs, including miR-1246, miR-98, miR-122 and miR-155, that regulate bone metabolism by targeting key signaling molecules. Specifically, miR-1246 downregulates GSK3B and Axin2; miR-98 suppresses DUSP-1; miR-122 inhibits Bcl-2 and miR-155 represses both BMP and SOCS6. Additionally, these exosomes modulate bone remodeling through the NF-κB signaling pathway. miRNAs/miRs, microRNAs; M1 Exo, M1 macrophage-derived exosomes; Omap, Office of Medical Assistance Programs; GSK3B, Glycogen synthase kinase 3 beta; DUSP-1, Dual specificity phosphatase 1; Bcl-2, B-cell lymphoma/Leukemia-2; BMP, Bone morphogenetic protein; Axin2, Axis inhibition protein 2; JNK, c-Jun N-terminal kinase; SOCS6, suppressor of cytokine signaling 6; BMMSC, Bone marrow mesenchymal stem cells; NF-κB, Nuclear factor kappa B.

exosome-derived miRNAs in bone formation and osteoclastogenesis remain to be fully elucidated (82). Emerging evidence suggests that interactions between BMMSCs and extracellular signals from M2 macrophages markedly contribute to bone formation. However, the exact mechanisms underlying these interactions remain debated, possibly due to differences in cell sources, co-culture conditions, and macrophage polarization protocols. Some studies propose that CM from M0 or M2 macrophages promotes the mineralization of BMMSCs in the later stages of osteogenesis (83-86). By contrast, other studies report opposing findings. For instance, Xia *et al* (73) found that exosomes derived from M2 macrophages inhibit BMMSC proliferation, with no significant effect on the expression of osteogenic or adipogenic genes in BMMSCs, and possibly even suppress chondrogenic differentiation. The regulatory effects of M2 macrophage-derived exosomes on bone remodeling signaling pathways are summarized in Fig. 7.

Effects of macrophage-derived exosomes on muscle growth and metabolism. Exosomes play a pivotal role in regulating protein synthesis and muscle regeneration by promoting satellite cell proliferation and myofiber formation. This regulation occurs through the delivery of myogenic growth factors such as IGF, FGF-2, and hepatocyte growth factor (HGF) (87). Forterre *et al* (88) employed proteomic analysis to identify numerous proteins associated with muscle growth and metabolism in exosomes secreted by skeletal muscle. For example, exosomes extracted from C2C12-derived myotubes contained key components, such as integrin subunit β1, CD9, CD81, neural cell adhesion molecule (NCAM), CD44 and myosin, that are potentially crucial for myocyte differentiation. Similarly, Mobley *et al* (89) demonstrated that exosomes derived from whey protein enhance muscle protein synthesis *in vitro*. Autophagy plays a critical role in maintaining muscle mass and function; its inhibition exacerbates muscle atrophy (90). AMPK, a central intracellular energy sensor, alleviates sarcopenia (SP) symptoms and mitochondrial dysfunction (91). Chen *et al* (92) discovered that exosomes from MSCs ameliorate muscle atrophy by enhancing AMPK/ULK1-mediated autophagy. Guescini *et al* (93) observed that exosomal miR-133b and miR-181a-5p are rapidly released into the bloodstream post-exercise, promoting muscle regeneration. This mechanism may partially explain how exercise mitigates SP. Furthermore, Chaturvedi *et al* (94) demonstrated in animal models that exercise-induced

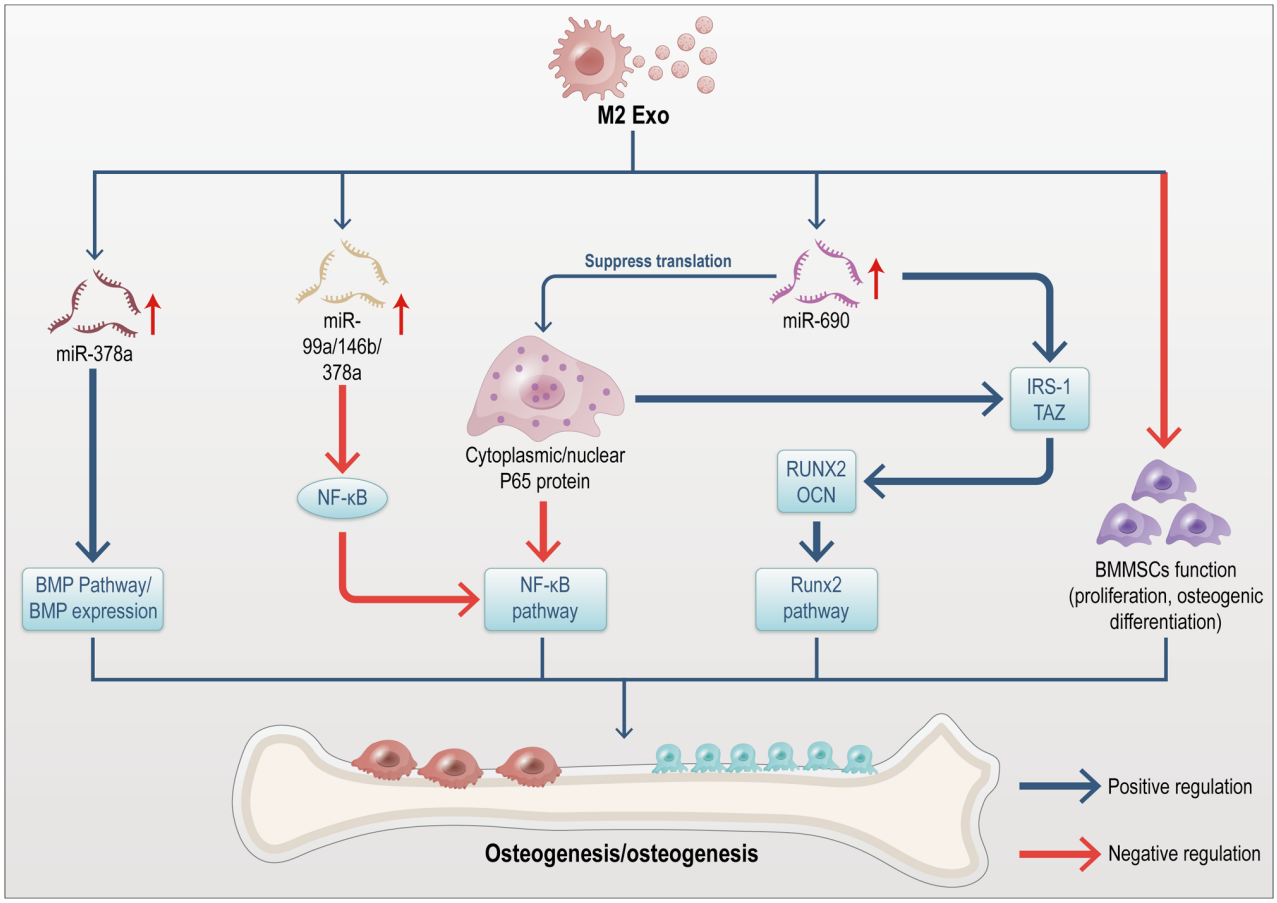

Figure 7. Regulation of bone remodeling signaling pathways by M2-derived exosomes. Exosomes derived from M2 macrophages carry miRNAs such as miR-378a, miR-99a, miR-146b, miR-378a, and miR-690, which positively influence bone metabolism. These miRNAs act through multiple targets, miR-378a enhances BMP signaling; the miR-99a/146b/378a cluster inhibits NF-κB; and miR-690 suppresses both NF-κB p65 and IRS-1 while promoting TAZ activity. Furthermore, M2 exosomes contribute to bone regulation by modulating the behavior of BMMSCs. miRNAs/miRs, microRNAs; BMP, Bone morphogenetic protein(s); NF-κB, Nuclear factor kappa B; IRS1, Insulin receptor substrate 1; OCN, osteocalcin; TAZ, Transcriptional co-activator with PDZ-binding motif; BMMSCs, Bone marrow mesenchymal stem cells.

exosomes promote skeletal muscle regeneration through the upregulation of miR-9b and miR-29.

Additionally, extracellular vesicles from M1 macrophages can polarize recipient macrophages toward an M2-like phenotype, thereby altering skeletal muscle homeostasis under high-glucose conditions. Collectively, these findings highlight the roles of both M1- and M2-derived macrophage exosomes in regulating SP.

The role of exosomes from macrophages in muscle-bone crosstalk. Exosomes serve as critical messengers in muscle-bone crosstalk, enabling skeletal muscles to influence bone activity through EV-mediated communication. This exosome-mediated communication between skeletal muscle cells occurs via autocrine, paracrine, or endocrine pathways (95-98). Exosomes derived from C2C12 myoblasts promote the osteogenic differentiation of MC3T3-E1 preosteoblasts, likely mediated by the upregulation of miR-27a-3p in recipient cells. This miRNA suppresses specific target genes and activates β-catenin signaling, thereby enhancing osteogenesis (99). Additionally, exosomes from C2C12 myoblasts enhance Wnt/β-catenin signaling in TOPflash-MLOY4 osteocyte-like cells, promoting cell survival and providing protection against apoptosis and oxidative stress. This protective effect is likely due to exosomes-mediated inactivation of Wnt inhibitors, such as SOST, DKK2, and SFRP2 (100). Li *et al* (101) showed that myoblast-derived exosomes carry Prrx2, which activates miR22HG transcription, promoting osteogenic differentiation of BMMSCs via the YAP pathway and miR-128. Furthermore, CXCR4, present on exosomes produced by mouse fibroblasts, targets these vesicles to the bone marrow, where miR-188-containing exosomes fuse with lipids to form hybrid nanoparticles. These nanoparticles then release miR-188 in a targeted manner, inhibiting adipogenesis and promoting BMMSC differentiation into osteoblasts (102). Regarding the influence of bone cell-derived exosomes on skeletal muscle, long non-coding RNAs (lncRNAs) are critical. For example, Zheng *et al* (103) found that osteoblasts induce myogenic differentiation of C2C12 myoblasts via exosomal lncRNAs, specifically TUG1 and DANCR. Toita *et al* (104) demonstrated that collagen patches releasing phosphatidylserine-containing liposomes promote M1-to-M2 macrophage polarization, facilitating concurrent bone and muscle tissue healing. Transcriptome analysis via next-generation sequencing revealed that M1 macrophage secretory products inhibit the differentiation of preosteoblasts and myoblasts, while M2 macrophage secretory products promote it. This highlights the importance of timely M1-to-M2 polarization for effective tissue regeneration. As shown in Fig. 8, macrophages,

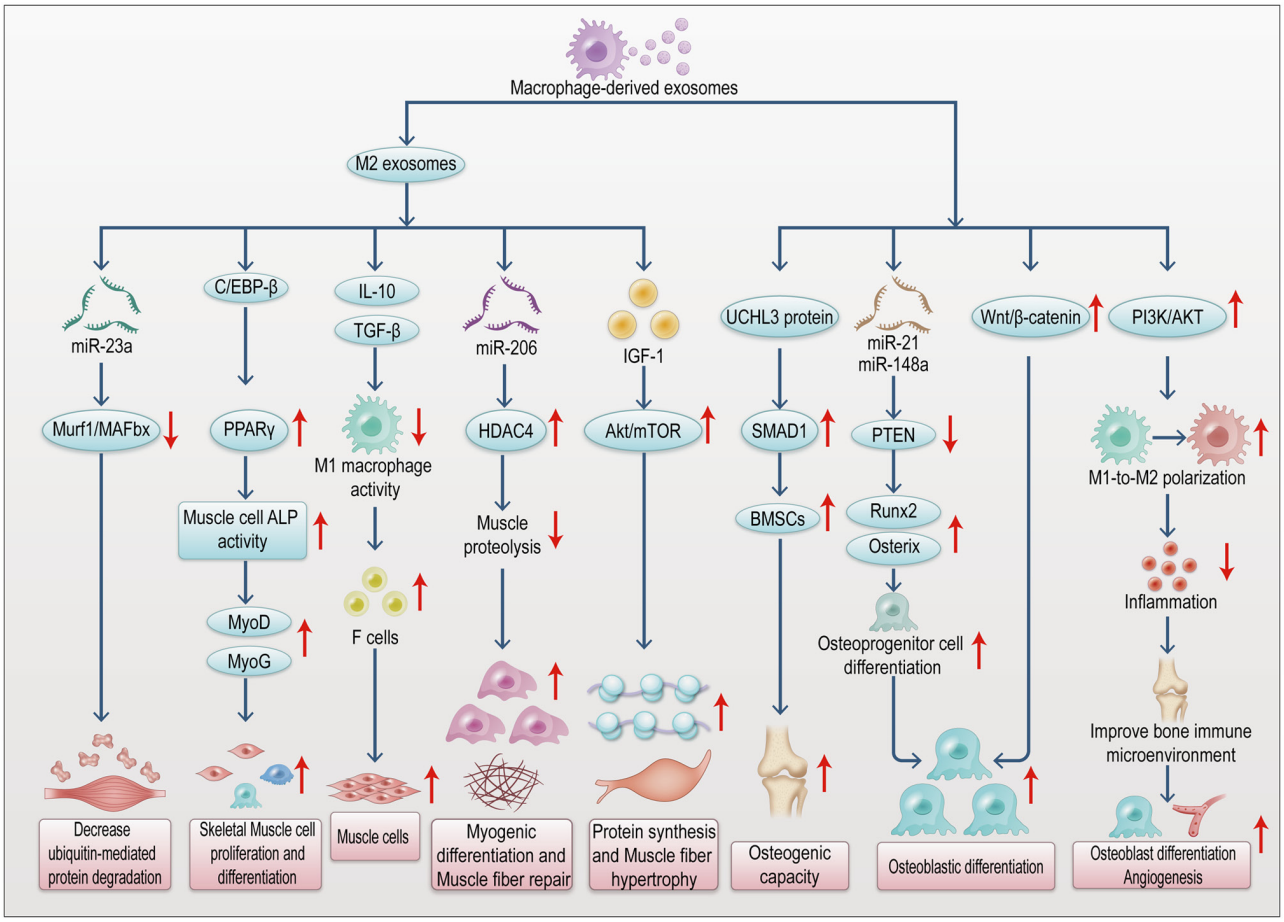

Figure 8. Mechanism of macrophage-derived exosomes on bone metabolism and musculoskeletal crosstalk. M2 macrophage-derived exosomes regulate muscle metabolism through several pathways, the miR-23a/MuRF1/MAFbx axis, C/EBP-β/PPARγ signaling, miR-206/HDAC4, Akt/mTOR signaling, and by modulating M1 macrophage activity. Concurrently, macrophage-derived exosomes regulate bone metabolism via the PI3K/Akt and Wnt/β-catenin pathways, miR-1481/PTEN and miR-21/PTEN axes, and the UCHL3/SMAD1 signaling cascade. miRNAs/miRs, microRNAs; PPARγ, Peroxisome proliferator-activated receptor gamma; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol-3-kinase; Akt, protein kinase B; MuRF1, muscle RING finger-1; MAFbx, F-box only protein 32; Smad1, SMAD family member 1; Wnt, wingless-type; MMTV integration site family; TGF-β, transforming growth factor beta; HDAC4, histone deacetylation 4; C/EBP-β, CCAAT-enhancer binding protein; UCHL3, ubiquitin carboxyl terminal hydrolase L3; ALP, alanine phosphatase; MyoD, myogenic differentiation 1; MYOG, Human myogenin; BMMSC, Bone marrow stromal cells; PTEN, Phosphatase and tensin homolog; RUNX2, Runt-related transcription factor 2.

particularly the M2 phenotype, release exosomes carrying the transcription factor C/EBPβ. This factor promotes skeletal muscle cell proliferation and differentiation via the PPARγ signaling pathway (105). Depletion of macrophage exosomes containing C/EBPβ markedly inhibits ALP activity in muscle cells and reduces the expression of myogenic markers (such as MyoD and MyoG), delaying muscle injury repair (105). M2 macrophage-derived exosomes suppress M1 macrophage activity by delivering anti-inflammatory factors, such as IL-10 and TGF-β, while mitigating the damaging effects of pro-inflammatory factors like TNF-α and IL-6 on muscle fibers. They also promote the directed differentiation of stem cells into myocytes (106). Additionally, M2 macrophage exosomes activate the PI3K/AKT pathway, facilitating the shift from M1 to M2 polarization. This process leads to reduced inflammatory cytokine levels, improved bone immune microenvironment and enhanced osteoblast differentiation and angiogenesis (107). Under mechanical stimulation, macrophages increase exosome secretion. These exosomes carry the UCHL3 protein, which enhances the osteogenic potential of BMMSCs and drives callus formation through the SMAD1 signaling pathway (108). Exosome-encapsulated miRNAs, including miR-21 and miR-148a, inhibit osteogenic inhibitors like PTEN and upregulate RUNX2 and osterix (106). In osteosarcoma models, exosomes coordinate osteoclast and osteoblast activity by transmitting Wnt/β-catenin signals, contributing to the maintenance of bone homeostasis (106). These findings highlight the critical role of macrophage-derived exosomes in regulating the proliferation and repair of both bone and muscle tissues, modulating muscle-bone crosstalk and enhancing tissue regeneration.

5. Mechanism of influence of macrophage-derived exosomes on bone metabolism of musculoskeletal crosstalk mediated by IGF-1 and FGF-2

Key molecules in macrophage-derived exosomes Regulation of IGF-1 and FGF-2 signaling by exosomal miRNA and protein components. Exosomes function as essential intercellular signaling vehicles, transporting a variety of biologically active molecules, including miRNAs, mRNAs, proteins and lipids (109). These components regulate bone metabolism by

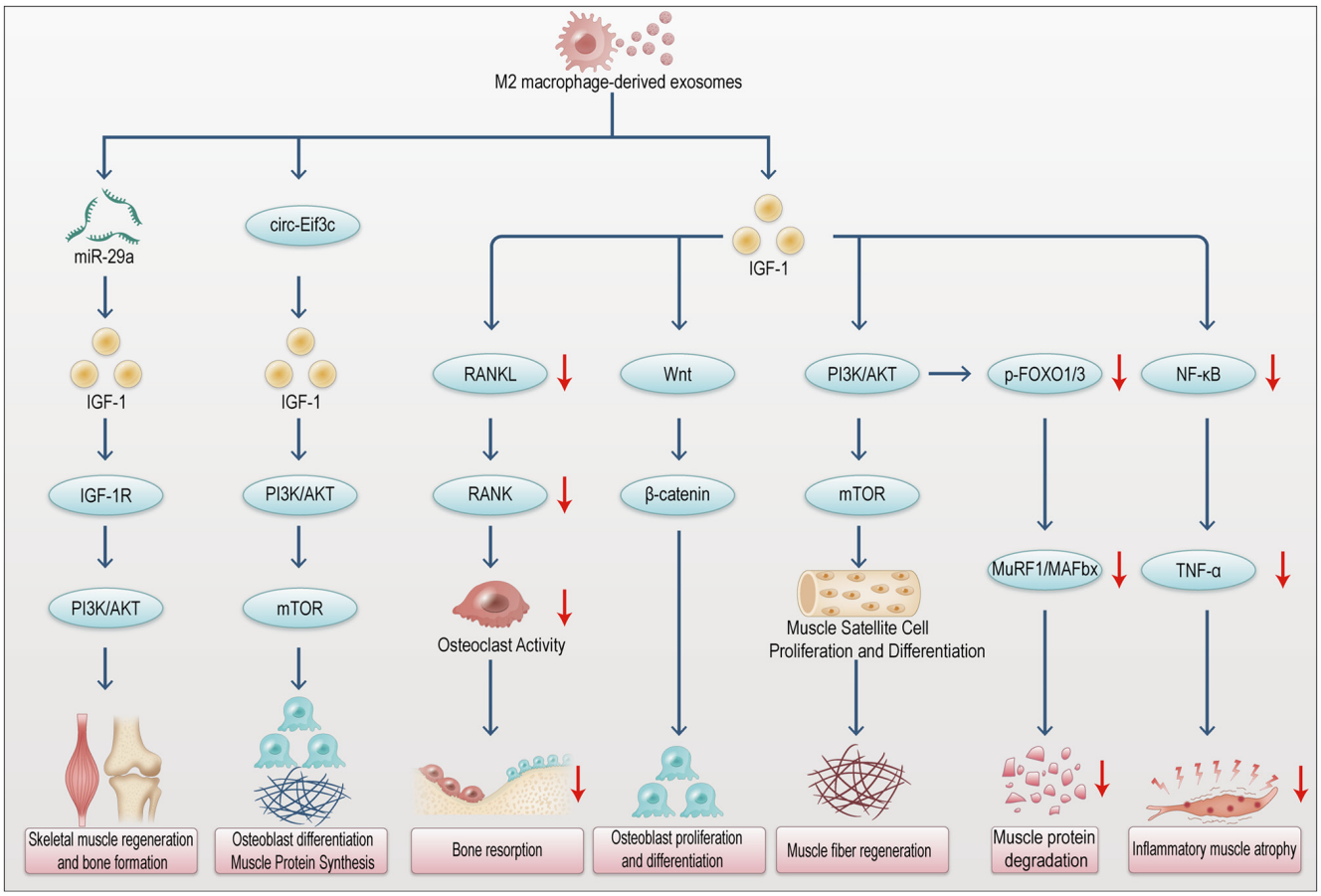

Figure 9. Mechanism of macrophage-derived exosomes on bone metabolism and musculoskeletal crosstalk by regulating IGF-1 expression. M2 macrophage-derived exosomes are enriched with miR-29a, circ-Eif3c, and IGF-1. miR-29a and circ-Eif3c regulate bone and muscle metabolism by modulating the IGF-1/PI3K/Akt signaling pathway. Independently, IGF-1 regulates muscle metabolism by inhibiting the MuRF1/MAFbx and NF-κB pathways. IGF-1, insulin-like growth factor 1; miRNAs/miRs, microRNAs; circ, circular RNA; PI3K, phosphatidylinositol-3-kinase; Akt, protein kinase B; MuRF1, muscle RING finger-1; MAFbx, F-box only protein 32; NF-κB, Nuclear factor kappa B.

modulating signaling pathways in target cells (110). Notably, miRNAs within macrophage-derived exosomes are recognized as key regulatory factors (111). Research indicates that macrophage-derived exosomal miRNAs influence bone metabolism indirectly by regulating key signaling molecules such as IGF-1 and FGF-2 (Figs. 9 and 10) (112). miRNAs modulate gene expression by binding to target mRNAs, resulting in translational repression or mRNA degradation (113). In musculoskeletal crosstalk, exosomal miRNAs from macrophages play pivotal roles in the regulation of IGF-1, FGF-2 and other signaling pathways (Figs. 9 and 10) (114). For instance, macrophage-derived exosomal miR-21 promotes IGF-1 signaling by targeting inhibitory molecules within the PI3K-Akt pathway, thereby enhancing bone formation (115). Similarly, macrophage-derived exosomal miR-29 boosts osteoblast function, promoting bone matrix synthesis and mineralization through the regulation of FGF-2 expression (116). By contrast, M1 exosomal miR-155 directly inhibits IGF-1R and downstream PI3K/Akt signaling, diminishing osteoblast differentiation (117). Additionally, M1 exosomal miR-143 exacerbates insulin-resistant muscle atrophy by inhibiting IRS-1 and obstructing the pro-muscle protein synthesis effect of IGF-1 (118). Thus, macrophage-derived exosomes regulate musculoskeletal crosstalk via exosomal miRNAs during IGF-1 and FGF-2 signaling, revealing a novel molecular mechanism (Figs. 9 and 10).

Interaction between exosome components and musculoskeletal cell surface receptors. Exosomes carry a diverse array of cargo molecules that regulate bone and muscle metabolism through interactions with receptors on the surface of musculoskeletal cells (119). Signal transduction by IGF-1 and FGF-2 in bone metabolism is initiated when these factors bind to their specific cell surface receptors (120). Macrophage-derived exosomes can modulate bone metabolism by transporting receptors or receptor ligands that facilitate the binding of signaling factors to their target cells (121). Exosomal ligands for IGF-1R and FGFR directly bind to their corresponding receptors on bone or muscle cells, triggering signal transduction (122). Upon IGF-1 binding, IGF-1R activates intracellular signaling cascades, primarily via the PI3K/Akt and MAPK pathways, to regulate bone and muscle growth and repair (123,124). Macrophage-derived exosomes can deliver IGF-1R or its ligands, thereby enhancing IGF-1 signaling to promote bone formation and muscle repair (Fig. 9) (125). Similarly, FGF-2 binding to FGFR initiates signaling cascades that regulate the proliferation and differentiation of bone marrow stromal cells and osteoblasts (126). FGF-2 targets sclerostin in bone and myostatin in skeletal muscle to counteract the harmful effects of glucocorticoids on musculoskeletal degradation (127). The exosome-mediated interaction between FGF-2 and FGFR enhances bone repair and plays a pivotal role in musculoskeletal

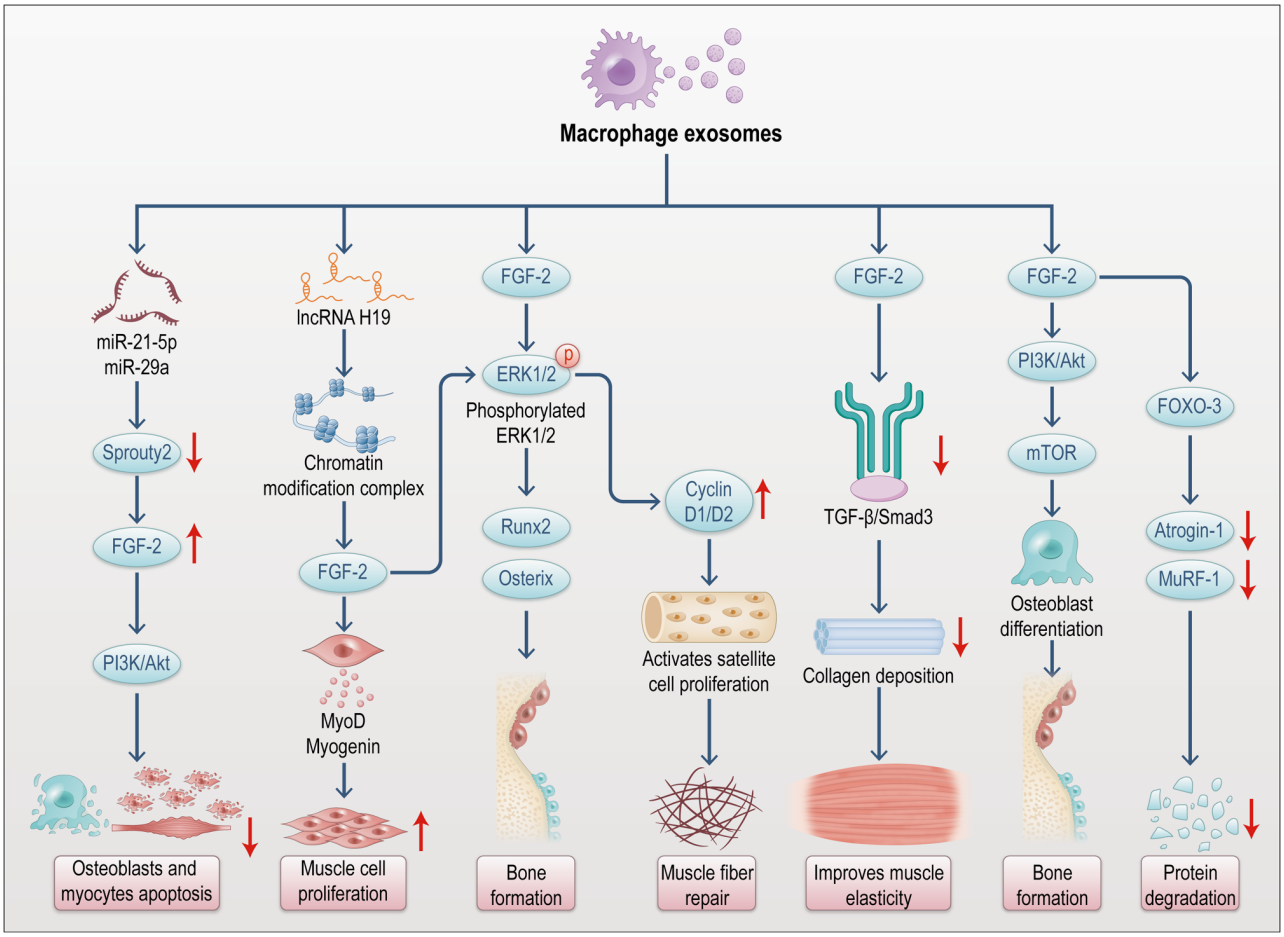

Figure 10. Mechanism of macrophage-derived exosomes on bone metabolism and musculoskeletal crosstalk by regulating FGF-2 expression. Macrophage exosomes contain miR-21-5p, miR-29a, lncRNA H19, and FGF-2. The miRNAs and lncRNA H19 regulate muscle metabolism by targeting FGF-2, activating PI3K/Akt signaling, and promoting the expression of myogenic factors MyoD and Myogenin. FGF-2 regulates both muscle and bone metabolism through the TGF-β/SMAD3, PI3K/Akt/mTOR, and FOXO-3 pathways. miRNAs/miRs, microRNAs; lnc, long non-coding; FGF-2, basic fibroblast growth factor; PI3K, phosphatidylinositol-3-kinase; Akt, protein kinase B; MuRF1, muscle RING finger-1; MyoD, Myogenic differentiation 1; ERK, extracellular regulated protein kinases; RUNX2, Runt-related transcription factor 2; TGF-β, transforming growth factor beta; SMAD3, Mothers against decapentaplegic homolog 3; mTOR, mammalian target of rapamycin; FOXO, forkhead box O; Atrogin-1, muscle atrophy-1.

crosstalk (Fig. 10) (128). In summary, macrophage-derived exosomes facilitate signal transduction and regulate bone metabolism by transporting receptors (such as IGF-1R, FGFR) and their ligands, strengthening bone formation and repair, and promoting musculoskeletal crosstalk.

The effect on osteoblast function

Regulating the proliferation, differentiation, and mineralization of osteoblasts. Osteoblast proliferation, differentiation and mineralization are essential processes in bone metabolism, regulated by macrophage-derived exosomes through various mechanisms (129). During osteogenesis, active molecules such as miRNAs and proteins facilitate osteoblast proliferation, differentiation, and mineralization (130). Exosomal miRNAs secreted by macrophages, including miR-21 and miR-29, can activate the PI3K/Akt and Wnt/β-catenin signaling pathways in osteoblasts, promoting their proliferation and differentiation (131); M1 macrophage-derived exosomes aggravate bone loss in postmenopausal osteoporosis via a miR-98/dual specificity phosphatase 1 (Dusp1)/c-Jun N-terminal kinase (JNK) axis (132); M2 macrophagy-derived exosomal miRNA-26a-5p induces osteogenic differentiation of bone mesenchymal stem cells (133); Exosomal miR-486-5p secreted by M2 macrophage influences the differentiation potential of bone marrow mesenchymal stem cells and osteoporosis (134). These miRNAs enhance osteoblast differentiation and bone matrix deposition by inhibiting negative regulatory factors and activating key transcription factors, such as Runx2 and Osterix (135,136). Additionally, exosomal proteins such as TGF-β and BMPs support mineralization by regulating osteoblast proliferation and differentiation (137). TGF-β activates the Smad signaling pathway through receptor binding, enhancing osteoblast mineralization (138). BMPs, on the other hand, activate the Smad1/5/8 pathway, which promotes osteoblast differentiation and stimulates bone matrix synthesis and mineralization (139), as summarized in Table V (140-143).

Affecting the expression of osteoblast-related genes (such as Runx2 and Osterix). The function of osteoblasts is further regulated by a series of transcription factors, with Runx2 and Osterix being two critical regulators of osteogenic differentiation (144). Macrophage-derived exosomes influence osteoblast function by modulating the expression of these transcription factors (145,146). Exosomes secreted by macrophages carry specific miRNAs, such as miR-124-3p and miR-146a, which

Table V. Regulation of the proliferation, differentiation, and mineralization of osteoblasts.

Authors, year	Active molecule	Mechanism of action	Signaling pathway/target	Effect	(Refs.)
Méndez-Mancilla *et al*, 2024	miR-21 miR-29	Promotes osteoblast proliferation and differentiation via pathway activation	PI3K/Akt, Wnt/β-catenin	Enhances osteoblast proliferation and differentiation	(140)
Fu *et al*, 2018	Runx2, Osterix	miRNA-mediated inhibition of negative regulators and activation of key transcription factors	Runx2, Osterix	Facilitates osteoblast differentiation and bone matrix deposition	(141)
Luo *et al*, 2017	TGF-β	Activates Smad signaling through receptor binding	Smad	Improves mineralization capacity of osteoblasts	(142)
Zou *et al*, 2021	BMP	Enhances bone matrix synthesis and mineralization via Smad1/5/8 signaling	Smad1/5/8	Promotes osteoblast differentiation and bone matrix mineralization	(143)

miRNAs, microRNAs; Runx2, regulation of Runt-related transcription factor 2; wnt, wingless-type MMTV integration site family; Runx2, Runt-related transcription factor 2; TGF-β, transforming growth factor-beta; BMP, bone morphogenetic protein; Smad, small mothers against decapentaplegic; PI3K, phosphatidylinositol 3-kinase; AKT, protein kinase B.

regulate Runx2 and Osterix expression (147,148). For example, miR-224-5p targets the 3' UTR of Runx2, preventing its degradation and thereby promoting osteoblast differentiation (149); miR-6879-5p carried by M2 macrophage-derived exosomes increases Runx2 expression and promotes osteogenic differentiation and aerobic glycolysis in human periodontal ligament stem cells (hPDLSCs) via modulating TRIM26-mediated ubiquitination of pyruvate kinase M (PKM) (150); M2 macrophage exosomes carrying miRNA-26a-5p can induce osteogenic differentiation of bone marrow-derived stem cells to inhibit lipogenic differentiation by promoting the expression of RUNX-2 (151). Additionally, miR-664-3p promotes osteoblast differentiation and mineralization by regulating Osterix expression (152). As a key transcription factor downstream of Runx2, Osterix drives bone matrix formation and mineralization (147). As well as miRNAs, proteins such as TGF-β and BMP in macrophage-derived exosomes also promote osteoblast differentiation by modulating Runx2 and Osterix expression (153,154). TGF-β enhances Runx2 expression through the activation of the Smad2/3 signaling pathway, promoting osteoblast differentiation (136). BMPs stimulate Osterix expression and enhance bone matrix deposition and mineralization via the Smad1/5/8 signaling pathway (155).

The effect on osteoclast function

Regulating the formation and activation of osteoclasts. The formation and activation of osteoclasts, the primary cells responsible for bone resorption, are essential processes in bone metabolism (156). Macrophage-derived exosomes contribute to bone resorption and regulate bone metabolism by modulating osteoclast formation and activation (157). Osteoclast formation is governed by various factors, including Receptor Activator of Nuclear Factor κ-B Ligand (RANKL) and Macrophage Colony-Stimulating Factor (M-CSF) (158,159). Macrophage-derived exosomes promote osteoclast formation by carrying RANKL and M-CSF (160,161). Specifically, exosomal RANKL binds to the RANK receptor on osteoclasts, activating the NF-κB and MAPK signaling pathways to drive osteoclast formation and activation (162,163). By binding to its receptor c-Fms, M-CSF activates the PI3K/Akt signaling pathway, accelerating osteoclast differentiation (164). Additionally, macrophage-derived exosomes can further promote osteoclast formation by modulating other cytokines, such as IL-1 and TNF-α, which enhance RANKL expression through the activation of the NF-κB signaling pathway, thereby promoting osteoclast activation (57). Consequently, macrophage-derived exosomes facilitate bone resorption by carrying RANKL, M-CSF, and other factors that regulate osteoclast formation and activation.

Changing the molecular mechanism related to osteoclast bone resorption activity. Osteoclast bone resorption activity is critical for regulating bone metabolism (165). Macrophage-derived exosomes modulate bone metabolism by influencing osteoclast resorption activity and altering underlying molecular mechanisms (166). The bone-resorbing capacity of osteoclasts heavily depends on the activation of surface receptors, particularly the RANKL/RANK ligand-receptor interaction (167). Macrophage-derived exosomes carrying RANKL can activate RANK receptors on osteoclasts, enhancing bone resorption activity (168). Moreover, exosomal matrix metalloproteinases (MMPs) contribute to bone resorption by degrading the bone matrix (169). Therefore, macrophage-derived exosomes augment osteoclast activity and bone resorption not only through the RANKL-RANK axis but also via MMPs and other molecules. Exosomal miRNAs and protein factors further regulate the molecular mechanisms of bone resorption by modulating enzymatic activities within osteoclasts (170). For instance, miR-146a promotes osteoclast activity by targeting negative regulators of the NF-κB pathway. By contrast, TGF-β enhances bone resorption by activating the Smad signaling pathway in osteoclasts (171). In summary, macrophage-derived exosomes play a pivotal role in regulating

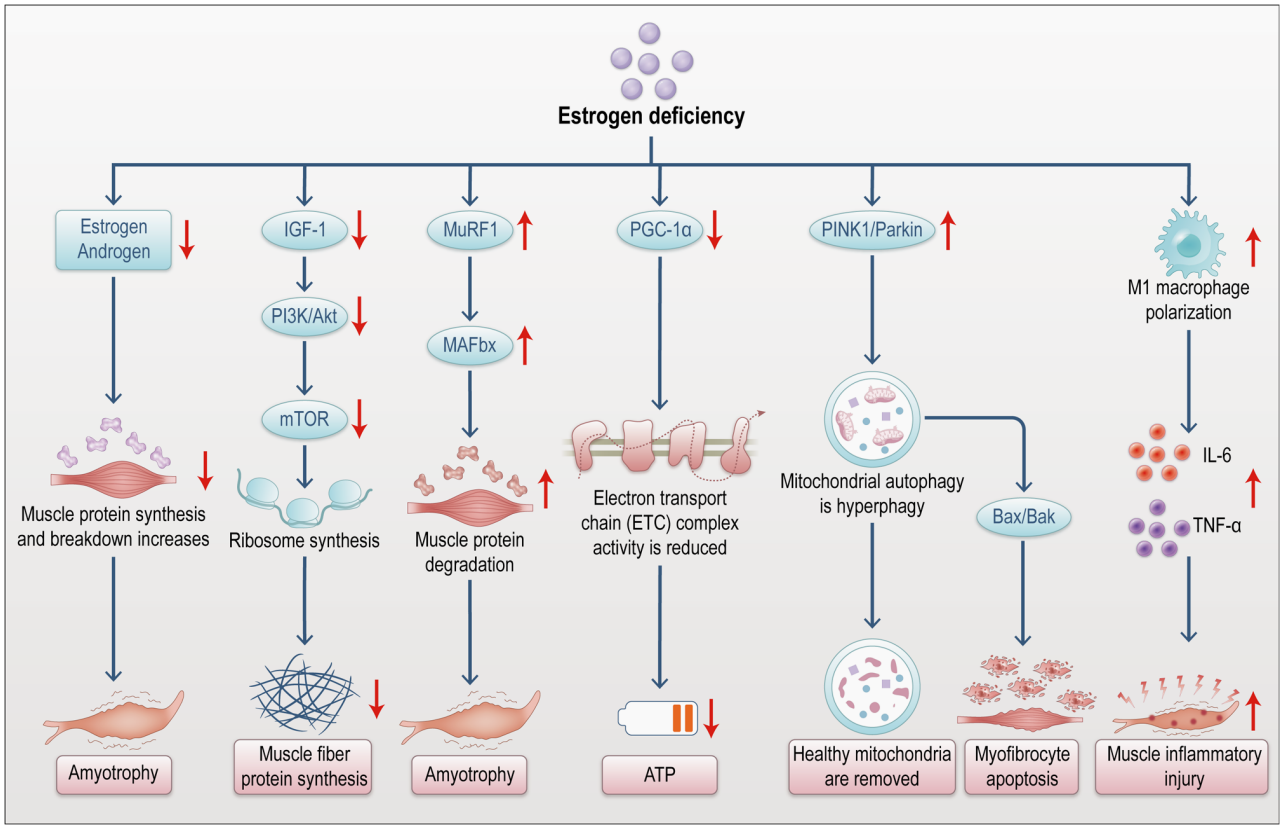

Figure 11. Regulatory mechanism of estrogen deficiency on bone metabolism during osteoporosis. Estrogen deficiency leads to reduced muscle synthesis and increased protein breakdown, resulting in muscle atrophy. Specifically, it decreases IGF-1 synthesis, inhibiting the PI3K/Akt/mTOR pathway and reducing muscle fiber protein synthesis. Concurrently, elevated MuRF1/MAFbx activity promotes muscle degradation. Impaired PGC-1α function reduces mitochondrial biogenesis, electron transport chain activity and ATP production. Increased PINK1/Parkin-mediated mitophagy clears dysfunctional mitochondria. Enhanced M1 macrophage activity contributes to muscle tissue inflammation. PI3K, phosphatidylinositol-3-kinase; Akt, protein kinase B; MuRF1, muscle RING finger-1; IGF-1, insulin-like growth factor 1; mTOR, mammalian target of rapamycin; PGC-1α, Peroxisome proliferator-activated receptor γ coactivator 1α; MAFbx, F-box only protein 32; PINK1, PTEN-induced putative kinase 1; ATP, Adenosine triphosphate; Bax, Bcl-2-associated X protein; BAK, BCL-2 antagonist killer 1.

bone metabolism by modulating the molecular pathways that control osteoclast-mediated bone resorption.

6. Function in pathological state

IGF-1 and FGF-2 play pivotal roles in both bone and muscle metabolism. Osteoporosis, often accompanied by SP, is a condition that involves diminished bone density and function, alongside muscle degradation. Macrophage-derived extracellular vesicles have been shown to improve both osteoporosis and SP by regulating IGF-1 and FGF-2 signaling.

Osteoporosis

Changes of macrophage-derived exosomes, IGF-1 and FGF-2 in the pathogenesis of osteoporosis. Osteoporosis is characterized by reduced bone density, deterioration of bone microarchitecture and increased fracture risk, primarily affecting older adults (172). Recent studies suggest that osteoporosis is not only associated with abnormal bone metabolism but also with impaired muscle function (Figs. 11 and 12), highlighting the significance of musculoskeletal crosstalk in the pathogenesis of osteoporosis (Figs. 11 and 12) (172,173). In osteoporosis, the functions of macrophage-derived exosomes, IGF-1, and FGF-2 are markedly altered. Macrophages, as key immune cells (174,175), contribute to bone metabolism through exosomes that transport a variety of signaling molecules, including cytokines, miRNAs, and proteins (176). Impaired macrophage function in patients with osteoporosis alters the signaling molecule profile within exosomes (177). These changes may accelerate bone loss by disrupting the balance between bone resorption and formation through various signaling pathways. IGF-1 plays a central role in bone metabolism by regulating bone formation, primarily through the PI3K/Akt and MAPK signaling pathways (178,179).

Influence of the interaction between the three on bone loss. The primary manifestations of osteoporosis, bone loss and reduced bone strength, are influenced by the interaction of macrophage-derived exosomes, IGF-1 and FGF-2 (180). These components synergistically regulate bone formation and resorption, thereby affecting bone mass (181). Exosomal miRNAs, such as miR-21 and miR-146a, derived from macrophages, can promote osteoclast differentiation and activity by modulating the RANKL/RANK pathway, leading to excessive bone resorption (182). Additionally, exosomal protein factors such as TGF-β and BMP further inhibit bone formation by regulating osteoblast function (183). In osteoporosis, IGF-1, a key osteogenic factor, is often underexpressed, impairing osteogenesis and leading to reduced bone formation (184). Similarly, FGF-2 signaling is suppressed in osteoporosis, contributing to further bone density loss (185). This disruption

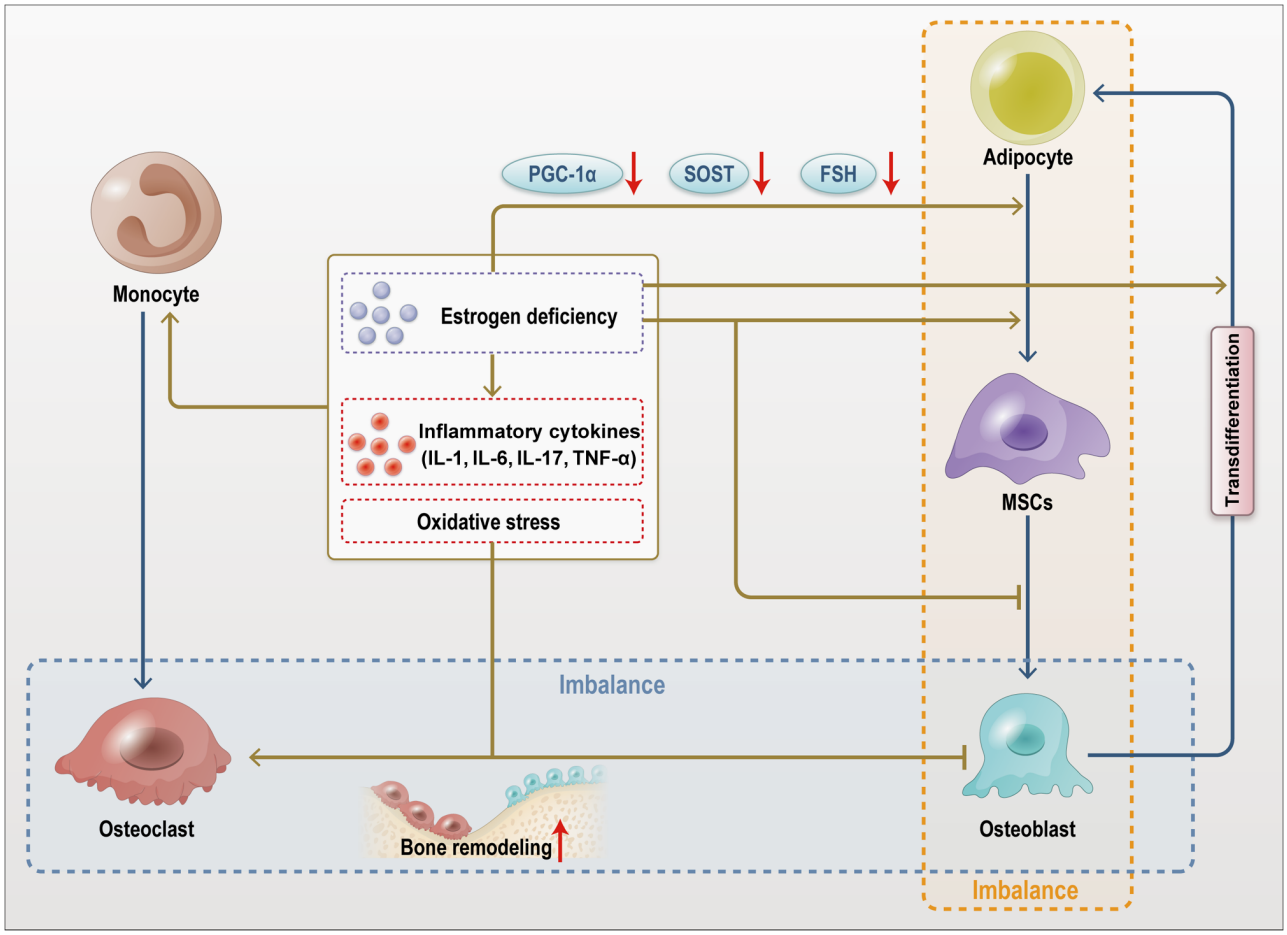

Figure 12. Regulatory mechanism of estrogen deficiency on muscle metabolism during osteoporosis. Estrogen deficiency disrupts bone remodeling by decreasing PGC-1α and elevating SOST and FSH, leading to increased inflammatory cytokines (such as IL-1, IL-6, IL-17 and TNF-α) and oxidative stress. These changes promote osteoclast differentiation and enhance bone resorption. Simultaneously, MSCs shift differentiation potential toward adipogenesis at the expense of osteogenesis. This imbalance between increased bone resorption and decreased bone formation results in impaired bone remodeling and net bone loss. PGC-1α, Peroxisome proliferator-activated receptor γ coactivator 1α; SOST, Sclerostin; FSH, Follicle-stimulating hormone; MSCs, Mesenchymal stem cells.

in signaling between IGF-1 and FGF-2 exacerbates bone loss. Exosomes carrying RANKL and TGF-β promote osteoclastogenesis and bone resorption (186), while the deficiency of IGF-1 and FGF-2 signaling further impairs bone formation. Disruption of this delicate musculoskeletal crosstalk and the associated molecular pathways plays a pivotal role in the pathogenesis of osteoporosis (187).

Abnormal bone metabolism associated with sarcopenia

Association between muscle atrophy and changes in bone metabolism in sarcopenia. SP refers to the age-related progressive loss of skeletal muscle mass and function (188). It is not only a natural part of aging but is also closely linked to various diseases and pathological conditions. The relationship between SP and abnormal bone metabolism has gained considerable attention in recent years. Muscle atrophy and bone metabolism interact bidirectionally, forming a critical regulatory mechanism for maintaining bone health (189). As depicted in Fig. 13, the onset of SP is often accompanied by decreased bone density and altered bone metabolism. Muscle atrophy influences bone metabolism through multiple mechanisms (190). It reduces muscle strength and load-bearing capacity, thereby diminishing mechanical stress on bones. This reduction in mechanical stimulation leads to increased bone resorption and decreased bone formation (190). Additionally, SP affects bone metabolism through the secretion of inflammatory factors, such as IL-6 and TNF-α (191). These inflammatory mediators activate osteoclast signaling pathways, enhancing bone resorption and consequently reducing bone density (192). Moreover, endocrine dysfunction linked to SP plays a significant role in abnormal bone metabolism. Muscle atrophy leads to reduced secretion of myokines, such as IGF-1 and FGF-2, impairing bone formation.

The role of macrophage-derived exosomes, IGF-1 and FGF-2 in this association. Macrophage-derived exosomes, along with IGF-1 and FGF-2, play pivotal roles in the bone metabolism abnormalities associated with SP (193,194). Macrophages, as key immune cells, secrete exosomes carrying a range of cytokines and miRNAs that are essential for musculoskeletal crosstalk (195). The miRNAs, cytokines and growth factors transported by macrophage exosomes affect all aspects of bone metabolism (196). For example, macrophage-derived exosomes promote osteoclast formation and activity by delivering miRNAs (such as miR-146a and miR-21), thus enhancing bone resorption (197). These exosomal miRNAs can activate specific signaling pathways by binding to receptors on bone cells, influencing the pathogenesis of conditions such as

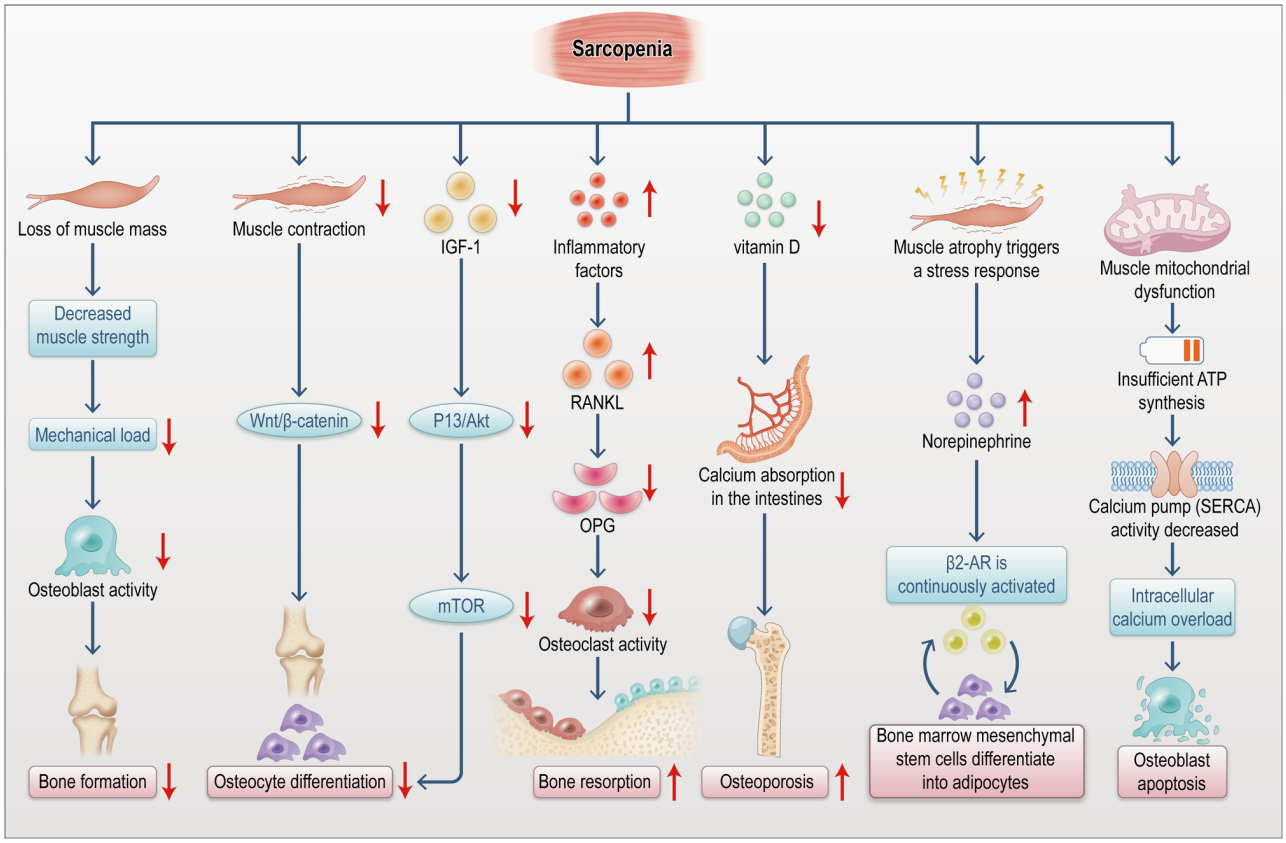

Figure 13. Regulatory mechanism of sarcopenia on bone metabolism during osteoporosis. Sarcopenia, characterized by the loss of muscle mass and reduced mechanical loading, leads to decreased osteogenic activity and bone formation. Impaired muscle contraction reduces Wnt/β-catenin signaling, inhibiting osteoblast differentiation. Declines in IGF-1 synthesis suppress PI3K/Akt/mTOR activity, further impairing osteoblast differentiation. Increased inflammatory cytokines elevate RANKL expression and suppress OPG, enhancing osteoclast activity and bone resorption. Reduced vitamin D intake decreases intestinal calcium absorption and bone formation. Muscle atrophy triggers a stress response, increasing norepinephrine production and sustained β2-AR activation, which promotes adipogenic differentiation of bone marrow mesenchymal stem cells. Muscle mitochondrial dysfunction reduces ATP synthesis, inhibits SERCA activity, causes intracellular calcium overload, and induces osteoblast apoptosis. PI3K, phosphatidylinositol-3-kinase; Akt, protein kinase B; IGF-1, insulin-like growth factor 1; mTOR, mammalian target of rapamycin; ATP, Adenosine triphosphate; RANKL, receptor activator of nuclear factor-κB ligand; OPG, osteoprotegerin.

osteoporosis and bone loss (197). Moreover, the roles of IGF-1 and FGF-2 in SP are particularly important (198). IGF-1 is well-established as essential for muscle tissue, promoting not only muscle growth and repair but also bone matrix formation by enhancing osteoblast function (199). However, circulating IGF-1 levels are often reduced in patients with SP, contributing to bone metabolism disorders. Similarly, FGF-2 is another key osteogenic factor involved in SP.

7. Research methods and technical means

Exosome isolation from macrophages typically involves techniques such as ultracentrifugation, size exclusion chromatography, immunoaffinity capture, and kit-based methods. Identification techniques include transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), western blotting (WB) and nanoflow cytometry. Each method offers distinct advantages and limitations. In muscle and bone metabolism experiments, *in vitro* methods include osteoblast differentiation assays, osteoclast inhibition tests, muscle cell differentiation experiments, and muscle metabolism studies. *In vivo* approaches include osteoporosis models, fracture healing models, and SP models.

Separation and identification of exosomes

Comparison and optimization of exosome separation techniques such as the ultrafast centrifugal method and the kit method. Exosomes are nanoscale extracellular vesicles (30-150 nm in diameter) secreted by cells and are widely distributed in various body fluids (200). The isolation and purification of exosomes from macrophages is a critical step in studying musculoskeletal crosstalk and its impact on bone metabolism (201). Conventional methods for exosome isolation primarily include ultracentrifugation and commercial kit-based approaches (201). Ultracentrifugation is regarded as the gold-standard technique for exosome separation, relying on density-based differentiation to isolate exosomes from other cellular components (202). This process involves removing cellular debris through low-speed centrifugation, followed by high-speed ultracentrifugation to pellet exosomes (202). Although this method is widely adopted, straightforward and cost-effective, it is time-consuming and requires significant technical expertise (202). Despite these limitations, ultracentrifugation remains the most commonly used method due to its high yield and efficiency in exosome collection (203). By contrast, commercial kit-based methods use reagents for exosome separation, often employing immunomagnetic

Table VI. Comprehensive overview of exosome isolation techniques.

Authors, year	Method	Principle	Application scenarios	Advantages	Limitations	(Refs.)
Coughlan *et al*, 2020	Ultracentrifugation	Separates exosomes based on density and size using high-speed centrifugation (100,000-200,000 x g)	Bulk exosome preparation; proteomic/ lipidomic analysis	High yield; no chemical additives required	Time-consuming (4-6 h); potential vesicle damage	(205)
Sidhom *et al*, 2020	Size-Exclusion Chromatography	Porous beads retain larger particles; exosomes elute first	Clinical studies; therapeutic development	High purity; maintains vesicle integrity	Low throughput; requires sample pre-filtration	(206)
Gao *et al*, 2022	Polymer precipitation (such as PEG)	Polymers reduce exosome solubility, inducing precipitation	Large-volume processing; initial screening	Simple protocol; cost-effective	Low purity (co-precipitates contaminants); polymer carryover	(207)
Greening *et al*, 2015	Immunoaffinity capture	Uses antibodies against surface markers (such as CD9, CD63, CD81)	Biomarker discovery; targeted isolation	High specificity; suitable for rare exosomes	Expensive (>$500/sample); antibody-dependent bias	(208)
Contreras-Naranjo *et al*, 2017	Microfluidics	Size- or charge-based sorting through nanochannels	Point-of-care diagnostics; small sample volumes	Rapid (30-60 min); portable systems available	Low yield (<50%); requires specialized equipment	(209)
Tang *et al*, 2017	Kit method	Utilizes immunomagnetic beads or affinity capture for isolation	High-purity exosome extraction from complex samples	Improved efficiency and purity	Higher cost; more complex operation	(210)
Huang *et al*, 2022	UC + kit method	Combines ultracentrifugation and kit-based strategies to mitigate limitations of each	Applications requiring balanced extraction efficiency and purity	Enhanced separation efficiency and purity	Increased cost and operational complexity	(211)

beads or affinity capture technologies (204). These kits are convenient, easy to use, and improve exosome purity markedly (204). However, they tend to be expensive and may exhibit lower extraction efficiency, especially in samples with high levels of contaminating cellular material (204). A comparative summary of these techniques is provided in Tables VI and VII (205-216).

Identification methods of exosomes (such as electron microscopy, WB detection of marker proteins). Exosome identification is a key aspect of exosome research (217). Commonly used characterization methods include electron microscopy, WB, and NTA (217). Electron microscopy is the most traditional approach for morphological characterization, allowing direct visualization of exosomes as spherical vesicles (212). Scanning electron microscopy (SEM) and TEM enable precise determination of exosome size, shape, and membrane structure (218). WB is routinely employed to identify exosomes by detecting specific marker proteins, including CD9, CD63, TSG101, and Alix (219). These proteins are highly enriched on the exosomal membrane and serve as canonical markers for exosome identification (219). Additionally, WB can analyze various exosomal cargoes (such as proteins, receptors and enzymes), offering insights into their biological functions in musculoskeletal crosstalk (220). NTA measures the size distribution of exosomes based on their Brownian motion, with most exosomes ranging from 30 to 150 nm in diameter (221).

Experimental cell model

Establishment of a co-culture system of muscle cells and bone cells. Investigating the impact of macrophage-derived exosomes, IGF-1, and FGF-2 on musculoskeletal crosstalk requires the establishment of a co-culture system that incorporates both muscle cells and osteocytes. Commonly used bone cell models include the MC3T3-E1 murine pre-osteoblastic cell line and the RAW264.7 murine monocytic cell line (222). These cell lines exhibit high proliferative and differentiation capacities, making them ideal for *in vitro* investigations (222). For muscle cell models, the C2C12 mouse myoblast cell

Table VII. Top 5 exosome isolation kits.

Authors, year	Name	Method	Sample Compatibility	Time	Yield	Purity	Price (US$)	(Refs.)
Huang *et al*, 2021	ExoQuick-TC™ (SBI)	Polymer precipitation	Serum, plasma, urine	30 min	High	Moderate	200-400	(212)
Lai *et al*, 2022	qEV2® (Izon Science)	Size-exclusion chromatography	Cell culture, CSF	1 h	Medium	High	300-600	(213)
D'Acunzo *et al*, 2022	Total Exosome Isolation™ (Thermo Fisher Scientific, Inc.)	Polymer-based precipitation	Biofluids, cell media	30 min	High	Low	250-450	(214)
Doyle *et al*, 2019	Exo-FETCH™ (Bio-Techne)	Anti-CD63/CD81 immunoaffinity	Small volumes (50-200 μl)	2 h	Low	Very high	500-800	(215)
Veerman *et al*, 2021	ExoEasy Maxi™ (Qiagen GmbH)	Membrane affinity	Large volumes (up to 4 ml)	1.5 h	High	Medium	400-700	(216)

CSF, cerebrospinal fluid.

line and the L6 rat skeletal muscle cell line are frequently used (223). The C2C12 cell line, in particular, differentiates into myotubes under appropriate conditions and is widely employed in muscle biology research (224). This co-culture model simulates the *in vivo* physiological environment and serves as a robust platform for studying muscle-bone interactions. Co-culture systems are typically established using either Transwell inserts or direct contact methods. In the Transwell system, a porous membrane (typically 0.4 μm) separates the cell types, enabling paracrine signaling via soluble factors while preventing direct cell-cell contact (225). This setup allows for precise control over the transfer of soluble factors between cell compartments (225). However, direct contact co-culture more accurately replicates the physical interactions and signaling processes, including mechanical stimulation, between muscle and bone cells (226).

Experimental designs to study the interaction of macrophage-derived exosomes, IGF-1 and FGF-2 in a cell model. Experimental designs investigating the interactions between macrophage-derived exosomes, IGF-1, and FGF-2 typically involve four key components: Exosome isolation and processing, gene interference, protein expression analysis and functional assays. A critical step in this experimental design is the processing of exosomes, typically secreted by macrophage cell lines such as RAW264.7. Exosomes from M1 macrophages are enriched with pro-inflammatory factors, while exosomes derived from M2 macrophages carry anti-inflammatory and tissue-repair factors (227). In experiments, exosomes isolated from macrophage cell lines (such as RAW264.7) are introduced into muscle and bone cell co-culture systems to assess their effects on cellular proliferation, differentiation, and mineralization (228). To determine the specific influence of IGF-1 and FGF-2, their functions can be inhibited using specific antagonists or gene interference techniques, such as siRNA, to confirm their roles in mediating musculoskeletal crosstalk. Additionally, techniques such as WB and qPCR can be employed to analyze how M1 and M2 exosomes regulate IGF-1 and FGF-2 expression, as well as the activity of their downstream signaling pathways, including PI3K/Akt and MAPK (229). Functional assays, such as osteogenic evaluation via Alizarin Red staining and osteoclastic activity assessment using TRAP staining, can be used to verify the effects of exosomes on bone cell function (230). Collectively, this experimental approach provides a framework for elucidating the complex interactions and regulatory mechanisms involving M1- and M2-macrophage-derived exosomes, IGF-1, and FGF-2 in bone metabolism.

Animal experimental model

Selection of suitable animal models (such as mice and rats) to simulate bone metabolism-related diseases. As summarized in Table VIII (231-239), animal models form the foundation for studying bone metabolism and related diseases. Commonly used mouse and rat models effectively simulate bone metabolic disorders and offer insights into the molecular mechanisms underlying bone metabolism; however, each model has distinct advantages and limitations. In osteoporosis research, ovariectomized mouse or rat models are frequently used to mimic the disruption of bone metabolism caused by estrogen deficiency (240). Additionally, drug interventions or genetic knockout techniques can establish disease models related to bone metabolism, allowing the investigation of the effects of exosomes, IGF-1, and FGF-2 (241,242). Bilateral ovariectomy is widely used to model postmenopausal osteoporosis (243), recapitulating key osteoporosis features, such as reduced bone mass and density (244). In rat models, glucocorticoid administration (such as dexamethasone) is commonly employed to induce abnormal bone metabolism, simulating drug-induced osteoporosis (245). These animal models provide valuable platforms for investigating the role of exosomes in bone metabolism.

Intervention methods and detection indicators of macrophage-derived exosomes, IGF-1, and FGF-2 in animal

Table VIII. Animal models for bone metabolism research.

Authors, year	Disease	Model	Induction method	Advantages	Limitations	Best applications	(Refs.)
Tenkumo *et al*, 2020	Osteoporosis	OVX mice/rats	Ovariectomy (estrogen loss)	Recapitulates human pathophysiology	Long modeling time (3-6 months)	Evaluating anti-resorptive therapies	(231)
Halloran *et al*, 2024		Aged C57BL/6 mice	Natural aging (≥18 months)	Spontaneous development	High individual variability	Studying age-related bone loss	(232)
Yashima *et al*, 2025	Fracture Healing	SD rat drill-hole	Surgical defect (1-2 mm)	Quantifiable healing process	Requires micro-CT monitoring	Biomaterial and scaffold evaluation	(233)
Muwanga *et al*, 2022		Mouse tibial fracture	Open fracture with fixation	Clinically relevant model	Technically challenging	Mechanical loading studies	(234)
Wang *et al*, 2019	Osteoarthritis	DMM mouse/rat	Medial meniscus destabilization	Progressive cartilage degeneration	Slow progression (8-12 weeks)	Disease-modifying drug testing	(235)
Suh *et al*, 2022		MIA rat	Sodium iodoacetate injection	Rapid induction (1-2 weeks)	Prominent acute inflammation	Anti-inflammatory agent screening	(236)
Li *et al*, 2024	Bone Tumors	Nude mouse OS model	Intratibial MG63 cell injection	Human-like tumor microenvironment	Lacks immune component	Metastasis/therapy studies	(237)
Ferrena *et al*, 2024		p53-KO transgenic mouse	Spontaneous osteosarcoma	Genetically relevant	Unpredictable tumor onset	Tumorigenesis mechanisms	(238)
Wang *et al*, 2024	Metabolic Bone Disease	HFD+STZ diabetic rat	High-fat diet + streptozotocin	Models diabetic osteopenia	Variable glycemic control	Glucose-bone interaction studies	(239)

OVX, ovariectomy; micro-CT, micro-computed tomography; SD, Sprague Dawley; p53 KO, p53 knock-out; OS, open source; DMM, destabilized medial meniscus; MIA, monosodium iodoacetate; MG63 cells, osteoblast-like osteosarcoma cells; HFD, high-fat diet; STZ, streptozotocin.

experiments. In animal studies, various intervention strategies are employed to investigate the effects of macrophage-derived exosomes (from M1 or M2 phenotypes), IGF-1, FGF-2 and related factors on bone metabolism. These agents, such as M1- or M2-derived exosomes, IGF-1 and FGF-2, are typically administered through local injection or systemic intravenous delivery. Researchers establish multiple experimental groups, including those treated with M1-derived exosomes, M2-derived exosomes, IGF-1, FGF-2, and vehicle controls, to enable direct comparison of the specific effects of each intervention on bone metabolism. Standard detection endpoints include bone densitometry such as dual-energy X-ray absorptiometry, histological analysis of bone tissue (such as hematoxylin and eosin [H&E] staining, Alizarin Red staining) and serum biomarkers of bone metabolism (such as osteocalcin, C-terminal telopeptide of type I collagen, bone-specific alkaline phosphatase) (246). These parameters offer a comprehensive evaluation of bone metabolism, allowing for an in-depth assessment of the effects mediated by M1 and M2 macrophage-derived exosomes. Collectively, these animal experiments shed light on how M1 and M2 macrophage-derived exosomes modulate IGF-1 and FGF-2 signaling within the musculoskeletal crosstalk network, influencing osteocyte proliferation, differentiation and mineralization. These findings provide a foundation for developing novel therapeutic strategies.

8. Effects of external factors on the osteogenic function of macrophage-derived exosomes

Diabetes increases the risk of delayed fracture healing and nonunion (247). Chen *et al* (242) found that alterations in the diabetic microenvironment induce functional changes in macrophages, leading to exosomes with markedly impaired osteogenic capacity when interacting with BMMSCs, compared with those from normal macrophages. *In vitro* assays demonstrate reduced mineralization and significant downregulation of osteogenic genes, consistent with *in vivo* findings (248). High blood sugar, insulin resistance, and inflammatory factors (such as TNF-α and IL-6) promote an increase in pro-inflammatory M1 macrophages (249). These

macrophages secrete exosomes rich in pro-inflammatory miRNAs (such as miR-155, miR-214 and miR-146) and inflammatory factors (250). For instance, miR-155-containing exosomes inhibit the Wnt/β-catenin signaling pathway, reducing osteoblast differentiation (251). M1 macrophage-derived exosomes, rich in pro-inflammatory factors like TNF-α and IL-6, activate the NF-κB pathway, inhibit Runx2 expression and suppress osteoblast differentiation (252). Moreover, M1 macrophage-driven exosomal miR-214 inhibits OPG, leading to an imbalance in the RANKL/OPG ratio and accelerating bone loss (253). Additionally, exosomal miR-146 inhibits IRS-1/Akt signaling, exacerbating insulin-resistant muscle atrophy (122). High blood sugar induces reactive oxygen species (ROS) accumulation, altering the protein and nucleic acid composition of macrophage-derived exosomes, including the presence of oxidative damage markers such as 8-OHdG (254). M1 exosomes containing ROS/glycation end-products (AGEs) can impair mitochondrial function, reduce ATP production and contribute to muscle weakness (255). Similarly, Song and Chung (256) demonstrated that exosomes from *Porphyromonas gingivalis*-infected macrophages inhibit MC3T3-E1 osteoblast proliferation, promote apoptosis, downregulate osteogenic gene expression and suppress osteogenic differentiation. Liu *et al* (257) observed that zinc ion concentration differentially modulates the osteogenic function of macrophage-derived exosomes. At 4 μmol/l $ZnCl_2$, macrophage-derived exosomes exhibited the strongest pro-osteogenic effects on osteoblasts, while at 20 μmol/l, they enhanced endothelial cell migration. Zhu *et al* (258) reported that magnesium ions (Mg^{2+}) promote macrophage autophagy and polarization, enhancing the pro-osteogenic effects of their exosomes on BMMSCs. Wei *et al* (259) showed that exosomes from BMP-2-stimulated macrophages markedly enhance osteogenesis. The osteogenic capacity of macrophage-derived exosomes is clearly influenced by external factors (260). Further research is needed to identify additional factors that modulate the osteogenic capacity of macrophage-derived exosomes, which holds important clinical implications.

9. The effect of sex differences on the role of estrogen in bone metabolism and muscle metabolism

Estrogen influences bone and muscle metabolism differently in males and females (Table IX) (261-271). The decline in estrogen levels is the primary cause of postmenopausal osteoporosis in women (240), leading to reduced bone mass, muscle atrophy and an increased risk of falls and fractures (240). SP often coexists with osteoporosis, resulting in osteosarcopenia (272). While male androgens predominantly regulate muscle and bone metabolism, estrogen remains crucial for maintaining bone strength and muscle metabolic adaptability (267). Further studies indicate that in obesity or infection models, male macrophages tend to be M1 polarized, with their exosomes rich in pro-inflammatory miRNAs (such as miR-155) that inhibit the insulin signaling pathway (273). By contrast, macrophage extracellular vesicles in female models carry higher levels of anti-inflammatory factors (such as miR-125a-5p), enhancing tissue repair (274). The decrease in estrogen levels in postmenopausal women leads to an M1/M2 imbalance, accelerating bone loss (with osteoporosis rates twice as high as in men) (274). In males, high testosterone levels are positively associated with muscle mass (275). However, obese males exhibit higher expression of miR-155 in macrophage exosomes from adipose tissue, which increases the risk of insulin resistance (276). M2 macrophage exosomes mediate the polarization of macrophages into anti-inflammatory phenotypes in female models, accelerating muscle regeneration (277). These findings suggest that estrogen exerts distinct effects on bone and muscle metabolism across sexes, leading to different regulatory mechanisms of macrophage exosomes in estrogen-induced muscle-bone metabolism abnormalities, influenced by sex-specific factors.

10. Challenge on macrophage exosomes in regulating musculoskeletal metabolism

Although RAB-GTPase-modified exosomes (RAB-EXOs), engineered via click chemistry, can target bone tissue in complex *in vivo* environments, their targeting and enrichment efficiencies remain suboptimal (278). Drug concentrations at sites of deep-seated bone infections, such as osteomyelitis, are often insufficient and systemic administration can result in off-target effects and potential adverse reactions. Studies show that intravenously injected exosomes are primarily taken up by the liver, skeletal muscle and adipose tissue (279-281). Enhancing exosome enrichment at specific bone lesion sites remains a critical challenge that needs urgent resolution (282). Exosomes derived from macrophages of different sources and polarization states exhibit significant differences in composition and biological function. Exosomes from M1 and M2 macrophages can exert opposing biological effects, and this heterogeneity presents a considerable challenge for developing standardized therapies. One study demonstrated that exosomes secreted by adipose tissue macrophages in obese mice promote insulin resistance (283), whereas those from M2 macrophages improve insulin sensitivity (284). Ensuring batch-to-batch consistency and functional stability of therapeutic exosomes is a major challenge, as is overcoming technical bottlenecks in their large-scale production. Exosomes isolated from 20-25 lean mice are needed to treat one obese mouse, yielding too little for clinical translation. Although *in vitro* induction of M2 macrophages can partially address source limitations, optimization of culture conditions, purification protocols and storage stability remains necessary. The use of composite technologies with carrier materials (such as hydrogels) also faces challenges in large-scale production. While specific miRNAs, such as miR-690, mediate the insulin-sensitizing effects of M2 macrophage-derived exosomes (284) and the PI3K/AKT pathway regulates macrophage polarization, significant knowledge gaps remain regarding their application in treating musculoskeletal tissues. The full molecular network underlying bone metabolic diseases is not yet fully understood (285). Key questions concerning exosomal release kinetics, interactions with host cells, and long-term effects of exosomal cargo require further in-depth investigation. This lack of knowledge hinders the precise design and optimization of therapeutic regimens.

Exosomes are nanoscale extracellular vesicles with diverse bioactivities; however, their long-term safety profile remains incompletely characterized. Although animal studies have

Table IX. Estrogen's key roles in bone density and muscle metabolism, sex-specific summary.

Authors, year	Function	Effects in Females	Effects in male	Primary mechanism	Clinical relevance	(Refs.)
Moura *et al*, 2018; Vilaca *et al*, 2022; Cauley 2015	Bone Density Regulation	• Maintains bone mass by inhibiting osteoclast activity (reducing bone resorption). • Promotes osteoblast proliferation and bone formation. • Rapid bone loss post-menopause due to estrogen decline (↑ fracture risk).	• Essential for maintaining bone mineralization via ERα/ERβ receptors. • Deficiency disrupts bone metabolism, leading to osteoporosis. • Aromatase converts testosterone to estrogen, indirectly protecting bones.	• Binds to estrogen receptors (ERα/ERβ) on bone cells. • Suppresses RANKL pathway, ↑ osteoprotegerin (OPG) to inhibit osteoclasts. • Enhances calcium absorption via vitamin D activation.	• HRT reduces postmenopausal osteoporosis risk. • SERMs (such as Raloxifene) mimic estrogen's bone-protective effects.	(261-263)
Pellegrino *et al*, 2022; Alexander *et al*, 2022; Jardí *et al*, 2018	Muscle metabolism	• Preserves muscle mass by reducing protein degradation. • Declines post-menopause contribute to sarcopenia (muscle loss) and ↓ strength. • Indirectly supports muscle function via mitochondrial stability.	• ESR1/ERα remodels mitochondrial cristae-nucleoid structure, ↑ metabolic resilience. • Enhances exercise adaptation and insulin sensitivity in skeletal muscle. • Low estrogen ↑ fat accumulation, ↓ muscle quality.	• Modulates mitochondrial biogenesis and oxidative metabolism. • Regulates insulin/IGF-1 signaling pathways. • Anti-inflammatory effects protect against muscle atrophy.	• Estrogen-based therapies may prevent sarcopenia in aging. • ERα activation counters metabolic diseases in males.	(264-266)
Unger *et al*, 2023; Collins *et al*, 2018; Tramunt *et al*, 2020; O'Reilly *et al*, 2021; Farhat *et al*, 2017	Key differences	Estrogen deficiency directly drives accelerated bone/muscle loss post-menopause.	Effects are subtler but critical for metabolic flexibility and musculoskeletal health.	Females: Dominant via direct receptor binding. Males: Relies on local aromatization of testosterone.	Screening for estrogen deficiency is vital in male osteoporosis.	(267-271)

IGF-1, insulin-like growth factor 1; ERα, estrogen receptor alpha; ERβ, estrogen receptor beta; SERMs; Selective estrogen receptor modulators; ESR1, estrogen receptor 1; OPG, osteoprotegerin; RANKL, receptor activator of NF-kappa B ligand; HRT, hormone replacement therapy; ERβ, estrogen receptor beta; SERMs, selective estrogen receptor modulators.

shown that RAB-EXOs induce no adverse reactions within a 28-day period in osteomyelitis treatment (286), their immunogenicity, potential toxicity and the risk of off-target effects in human applications require systematic evaluation. This is especially important for patients with metabolic diseases, as exosome-based therapies may have complex and unpredictable effects on systemic metabolic networks. Consequently, a comprehensive safety evaluation framework and robust risk mitigation strategies must be established.

11. Conclusion

In conclusion, exosomes derived from M1 and M2 macrophages play pivotal roles in muscle-bone crosstalk, particularly within molecular signaling pathways mediated by myokines such as IGF-1 and FGF-2. These factors are essential regulators of muscle growth and bone metabolism, promoting muscle cell proliferation and differentiation while influencing bone cell function through various signaling pathways. Their roles extend beyond direct regulation of local cellular behavior, modulating bone metabolic homeostasis via macrophage-derived exosomes. Ongoing research continues to uncover the complex mechanisms through which M1 and M2 macrophage-derived exosomes affect bone metabolism. Exosomes facilitate intercellular communication by transporting bioactive molecules (such as proteins, RNAs and lipids), regulating bone tissue formation and remodeling. Specifically, exosomes secreted by M1 and M2 macrophages can modulate bone density and strength by influencing osteoblast and osteoclast activity, promoting bone health and aiding in repair. These findings provide valuable insights into the intricate mechanisms governing bone metabolism and lay the groundwork for developing new therapeutic strategies.

12. Future development

To address current challenges, researchers are exploring several strategies, including engineering targeted modification technologies to enhance exosomal tissue specificity, establishing standardized production and quality control protocols, applying multi-omics approaches to clarify mechanisms of action and developing smart, responsive carrier systems to improve delivery efficiency. Innovative solutions, such as M2 macrophage exosome-hydrogel composites, show promise for bone regeneration therapy, but further preclinical and clinical studies are essential to evaluate their safety and efficacy. Interdisciplinary collaboration will be vital in advancing this field. Further investigation into the mechanisms of M1 and M2 macrophage-derived exosomes is expected to lead to more effective interventions for bone-related diseases, including osteoporosis and fracture repair.

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Availability of data and materials

Not applicable.

Authors' contributions

Conceptualization was by RMC, MZ, and JBH. Data curation was by ZXW and YFC. Funding acquisition was secured by MWL. Investigation was by SJG and YLZ. Project administration was performed by YLZ. Software management was overseen by ZBY. Figures 1-13 were prepared by MWL and RMC. Supervision was led by MWL. Validation was performed by RMC and visualization was by MZ. The original draft of the manuscript was written by MWL, who contributed to the review and editing. Data authentication is not applicable. All authors read and 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.

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