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Emerging Role of Extracellular Vesicles in Bone Remodeling

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Abstract

Extracellular vesicles (EVs), as nanometer-scale particles, include exosomes, microvesicles, and apoptotic bodies. EVs are released by most cell types, such as bone marrow stem cells, osteoblasts, osteoclasts, and immune cells. In bone-remodeling microenvironments, EVs deliver specific proteins (e.g., tenascin C and Sema4D), microRNAs (e.g., miR-214-3p, miR-183-5p, and miR-196a), and other growth factors (e.g., bone morphogenetic protein 1 to 7 and transforming growth factor β1) to osteoblasts and regulate bone formation. In addition, EVs can deliver cytokines, such as RANK (receptor activator of nuclear factor κB) and RANKL (RANK ligand), and microRNAs, such as miR-218 and miR-148a, to modulate osteoclast differentiation during bone resorption. EVs also transfer bioactive molecules and have targeted therapies in bone-related diseases. Moreover, bioactive molecules in EVs are biomarkers in bone-related diseases. We highlight the emerging role of EVs in bone remodeling during physiologic and pathologic conditions and summarize the role of EVs in tooth development and regeneration. At the end of this review, we discuss the challenges of EV application in the treatment of bone diseases.

Keywords: exosomes, microRNAs, osteoblasts, osteoclasts, osteoporosis, regeneration

Introduction

Bone remodeling is vital for maintaining the balance of bone metabolism. Bone remodeling involves bone-related cells, such as osteoblasts, which are responsible for bone formation; osteoclasts, which are specialized for bone resorption; mechanosensitive osteocytes, which are embedded in the bone matrix; and bone marrow stem cells (BMSCs), which differentiate into osteoblasts (Lee et al. 2017). In addition to bone-related cells, other cellular systems—such as immune cells (T cells, dendritic cells [DCs], and monocytes) and articular cartilage—exert important roles in bone remodeling. Intercellular communications among these cells are important for bone remodeling (Park et al. 2017). One way that these communications occur is through direct contacts via adhesion and juxtacrine interactions to transmit signals and modulate physiologic activities. Another way is via soluble mediators circulating in body fluids. One of the best-established and widely accepted models of soluble mediators is the RANK (receptor activator of nuclear factor kappa B) / osteoprotegerin / RANKL (RANK ligand) system. This system indicates communication between osteoblasts and osteoclasts.

Recent studies documented that extracellular vesicles (EVs) act as mediators in intercellular communications (Pitt et al. 2016). EVs are released by most cell types and circulate in blood, urine, and cell medium (Théry et al. 2009). Based on size, morphology, and biological features, EVs are classified into exosomes, microvesicles (MVs), and apoptotic bodies (Table 1). Each form of EV has its own formation and secretion (Fig. 1). Exosomes are derived from endosomal multivesicular bodies, which fuse with the plasma membrane (Cocucci and Meldolesi 2015). The endosomal sorting complex required for transport and the Rab family play crucial roles in the formation and release of exosomes (Théry et al. 2009). MVs are generated by outward budding from the plasma membrane. Apoptotic bodies appear only when cells are in the late stage of apoptosis.

We highlight the emerging role of EVs in bone remodeling during physiologic and pathologic conditions and summarize the roles of EVs in tooth development and regeneration. At the end of the review, we discuss the challenges of EV applications in the treatment of bone diseases.

Bioactive Cargoes and Characteristics of Bone-Related EVs

EVs carry several kinds of cargoes, such as proteins, RNA, DNA, and lipids, when secreted into extracellular microenvironments (Cocucci and Meldolesi 2015). The cargo of bone-related EVs

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Table 1. Characteristics of Different Types of Extracellular Vesicles.

Characteristic	Microvesicles	Exosomes	Apoptotic Bodies
Size, nm	50 to 1,000	30 to 150	50 to 5,000
Morphology	Heterogeneous	Homogeneous (cup shape by TEM)	Heterogeneous
Parental cell condition	Physiologic conditions or in response to stimuli	Physiology and pathologic conditions	Apoptosis
Formation mechanism	Plasma membrane	Endocytic pathway	Plasma membrane
Release pattern	Shedding or budding	Fusion of the membrane of multivesicular bodies with the plasma membrane	Outward blebbing
Cargo	Several lipids and phosphatidylserine contain membrane components similar to those of the parental cell membrane	Proteins, DNAs, RNAs, and lipids similar to those in the parental cells	Cell organelles, DNA fragments, and RNA
Markers	Integrins, MMPs, CD40	CD63, CD83, Alix	Caspase 3, histones

MMP, matrix metalloproteinase; TEM, transmission electron microscopy.

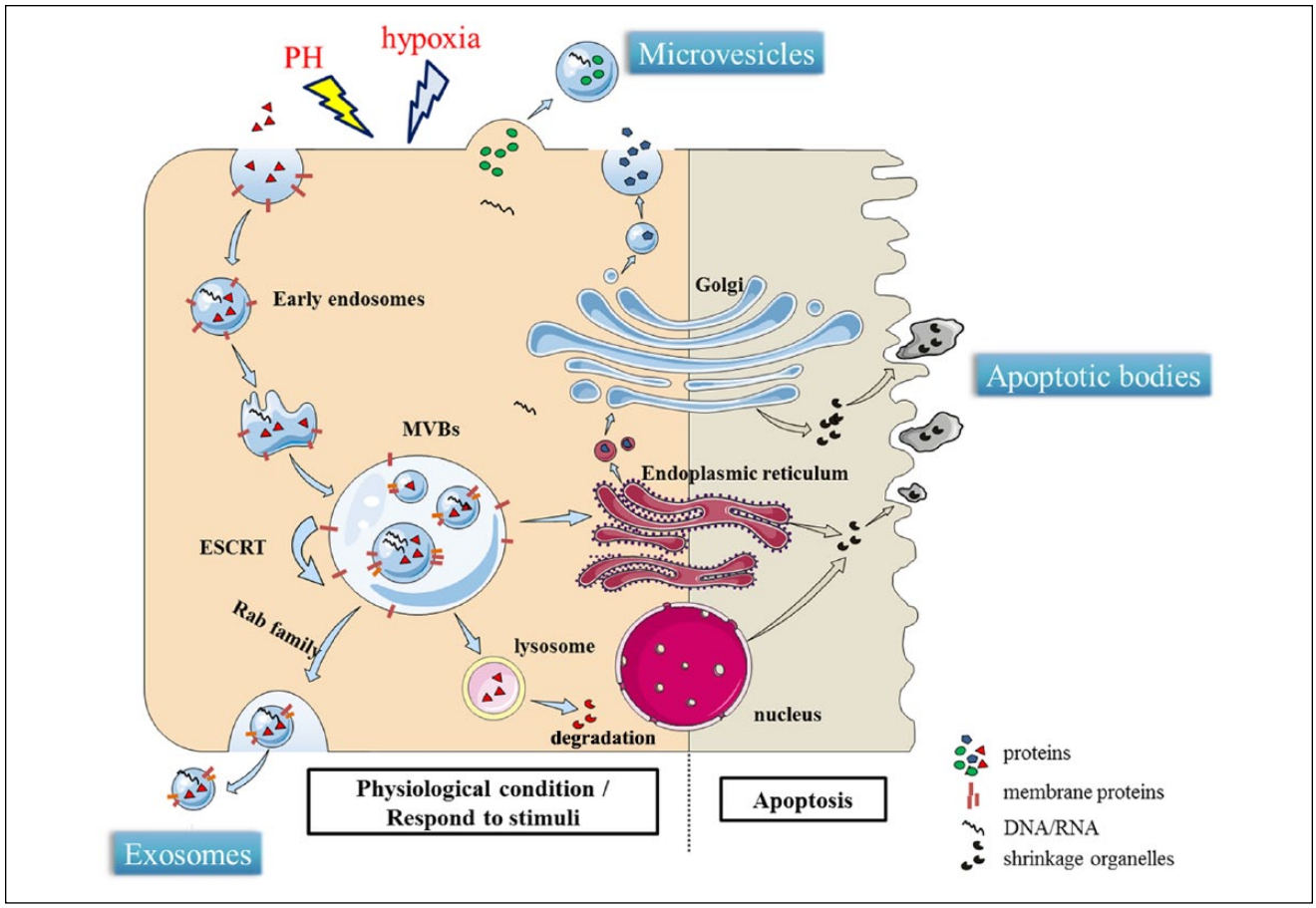

Figure 1. Biogenesis and secretion of different types of extracellular vesicles. Microvesicles are formed when the plasma membrane directly buds. Exosomes are derived from multivesicular bodies (MVBs) and secreted by fusion of MVBs with the plasma membrane. Apoptotic bodies containing organelles appear only when cells undergo the late stage of apoptosis. Changes of microenvironment, such as pH and hypoxia, can affect the secreting rhythm of extracellular vesicles. ESCRT, endosomal sorting complex required for transport.

can be classified into canonical and special species (Fig. 2). Canonical species—such as cytoskeletal proteins (tubulin, actin), membrane trafficking proteins (annexins, Rabs), multivesicular body formation (TSG101, Alix), tetraspanins (CD63, CD9, CD83), specific stress proteins (heat-shock proteins, HSP60, HSP70, HSP90), and enzymes (GAPDH, ATPase)—are closely associated with vesicle trafficking and biogenesis (Baglio et al. 2015).

Special cargo in EVs reflects the function of parent cells. In the bone-remodeling microenvironment, bone-derived EVs contain specific osteogenetic proteins, such as bone morphogenetic protein 1 to 7 (BMP1-7), alkaline phosphatase (ALP), and

eukaryotic initiation factor 2, as well as non-collagenous matrix proteins, such as bone sialoprotein (BSP), osteopontin (OPN), osteocalcin (OCN), and osteonectin (ON) (Xiao et al. 2007). EVs also contain proteins related to osteoclast differentiation, such as RANKL and RANK (Deng et al. 2015; Huynh et al. 2016). There are bone-related microRNAs (miRNAs) in EVs, such as miR-24, let-7, miR-143-3p, miR-10b-5p, miR-199b, miR-218, and miR-214-3p. These miRNAs play important roles in osteoblastic differentiation (Li et al. 2016; Qin et al. 2017). The number and distribution of miRNAs in exosomes are not consistent with those in exosome-producing cells (Baglio et al. 2015). It is speculated that miRNAs are selectively loaded into exosomes and transport specific information. There are also many kinds of mRNAs, such as those involved in the regulation of transcription (BDP1, TAF7 L, and SOX11) and kinase activity (LPAR1 and ZEB2) in bone-derived EVs (Morhayim et al. 2017). Some types of cargo, such as proteins and miRNAs in EVs, are annotated in EV databases (ExoCarta and Vesiclepedia).

Physiologic changes and chemical stimuli could influence the release rhythm and contents of EVs in bone remodeling. Early-passage primary BMSCs release fewer exosomes than those from later passages (Baglio et al. 2015). The species of miRNAs in exosomes from the human BMSCs in osteogenetic induction are different from those in the noninduction stage (Xu et al. 2014). Besides, age also influences the miRNA profile in EVs. EVs from the bone marrow interstitial fluid of young mice show higher expression of the miR-183 cluster (miR-96/-182/-183) than those isolated from aged mice (Davis et al. 2017). In addition, EV release rhythm is influenced by pathologic conditions, such as inflammation, hypoxia, and pH (Logozzi et al. 2009; Ayers et al. 2015). Some molecules, such as 1α,25-(OH)2D3 and imipramine, also regulate MV production and/or maturation (Woeckel et al. 2010; Deng et al. 2017).

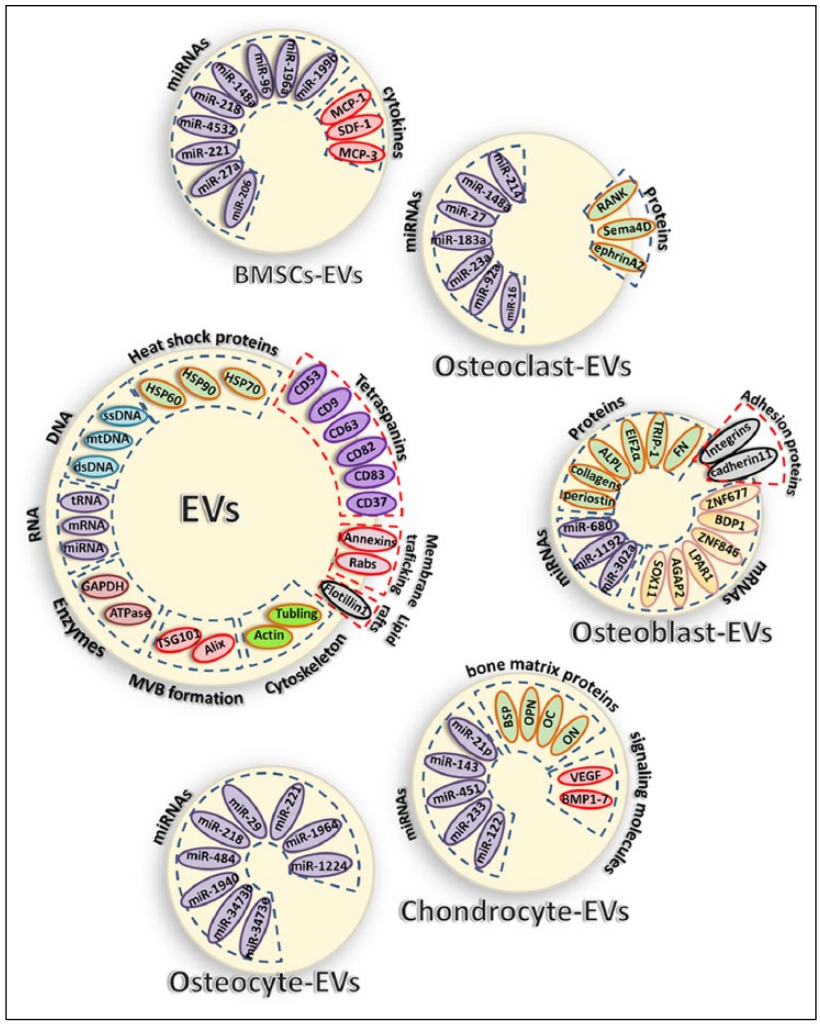

Figure 2. Canonical and special components of bone-related extracellular vesicles (EVs). This sketch illustrates the components of typical EVs. Bone marrow stem cells (BMSCs)–EVs contain miRNAs related to osteogenetic differentiation (e.g., miR-196, miR-27a) and cytokines (e.g., MCP-1, SDF-1). Osteoblast-EVs contain proteins (collagens, TRIP-1), miRNAs (miR-1192, miR-680, et al.), mRNAs (AGAP2, ZNF677), and adhesion proteins (integrins, cadherin 11). Osteoclasts-EVs contain proteins (RANK, ephrinA2, Sema4D) and miRNAs (miR-214, miR-148a-3p). Osteocyte-EVs are reported to contain some miRNAs (e.g., miR-218). Chondrocyte-EVs contain many miRNAs (e.g., miR-122-5p, miR-223-3p), signaling molecules (e.g., BMP-1-7, VEGF), miRNAs (e.g., miR-122-5p, miR-223-3p), and bone matrix proteins (e.g., bone sialoprotein [BSP], osteopontin [OPN]). MVB, multivesicular body. FN, fibronectin; OCN, osteocalcin; ON, osteonectin; RANK, receptor activator of nuclear factor kappa B; TRIP-1, transforming growth factor beta receptor II interacting protein 1.

Role of EVs in Cellular Communication in Bone Remodeling

EVs derived from BMSCs, osteoblasts, osteoclasts, osteocytes, and immune cells target adjacent cells and play important roles in cellular communication in bone remodeling (Fig. 3; Table 2).

EVs in BMSC-BMSC Communication

BMSC-secretome is an important part of the therapeutic effect of BMSCs in bone regeneration and radiation damage repair. The EVs of transplanted BMSCs can "drive" the functions of adjacent BMSCs. Exogenous BMSC-EVs are taken up and promote the osteogenic differentiation of endogenous BMSCs (Liu et al. 2015). Exosomal miR-151-5p from BMSCs targets endogenous BMSCs to rescue impaired ontogenetic differentiation and reduce adipogenic differentiation (Chen et al. 2017). Radiotherapy is a common treatment for malignancies

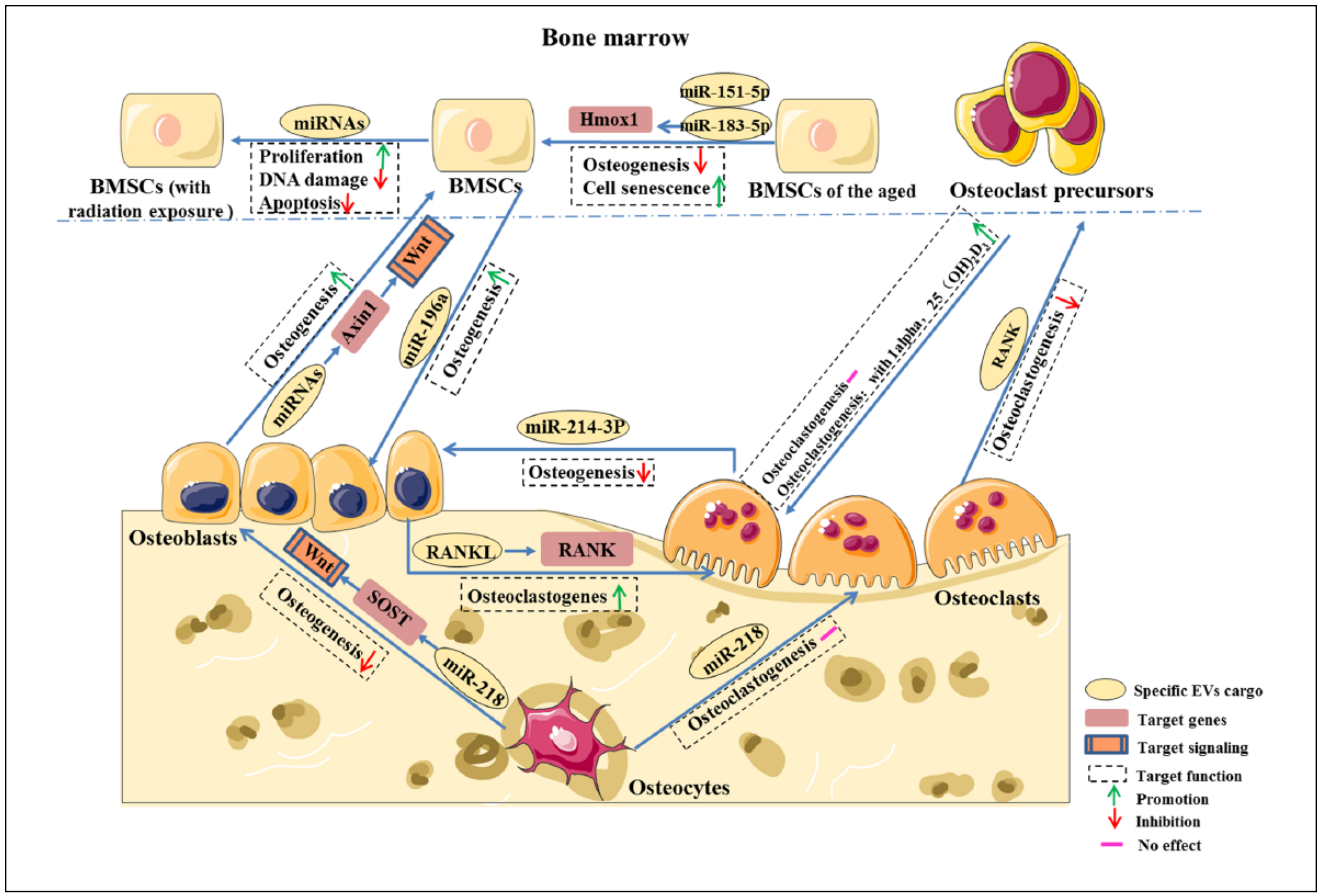

Figure 3. Signal networks of bone-derived extracellular vesicles (EVs) in regulation of bone remodeling. Bone marrow stem cells (BMSCs), osteoblasts, osteoclasts, and osteocytes communicate with one another via EVs. BMSCs-EVs inhibit DNA damage and apoptosis in irradiated hematopoietic cells and promote proliferation of bone marrow stromal cells by miRNA delivery. EVs from bone marrow fluid in old mice could inhibit osteogenic differentiation and increase the cell senescence of BMSCs in young mice. BMSCs-EVs could also accelerate osteogenic differentiation through miR-196a in osteoblasts. Osteoblasts could promote osteoblastic differentiation of BMSCs through miRNAs in EVs. Osteocytes inhibit osteoblastic differentiation by the lower level of miR-218 in EVs, which is regulated by muscles. Osteoblasts also modulate osteoclast differentiation by exosomal RANKL. EVs from osteoclasts inhibit osteoblastic differentiation by miR-214-3p delivery. Osteoclast-EVs regulate the osteoclastic differentiation.

and exerts harmful effects on the bone marrow. A new study found that normal BMSC-derived EVs could promote proliferation and decrease DNA damages of BMSCs after radiation exposure (Wen et al. 2016).

EVs in Bidirectional BMSC-Osteoblast Communication

BMSCs play a vital role in osteoblastic differentiation via the paracrine pathway (Santos et al. 2015). Recent studies have demonstrated that when cocultured with human osteoblasts (hFOB 1.19), PKH67-labeled BMSC-EVs are distributed in organelles, such as the endoplasmic reticulum, Golgi apparatus, and lysosomes of these osteoblasts. This finding indicates that BMSC-derived EVs could be endocytosed by osteoblasts and release the cargo into the target cells (Qin et al. 2016). Moreover, the role of BMSC-derived EVs in bone remodeling has been illuminated in vivo. When human BMSC-derived exosomes are delivered to the calvarial-defect areas of Sprague-Dawley rats, these exosomes promote bone regeneration (Qin et al. 2016).

In turn, osteoblasts send messages to BMSCs via EVs. Exosomes derived from mineralized osteoblasts (MC3T3-E1 cells) can be absorbed by bone stromal cells (ST2 cells). These exosomes promote osteoblastic differentiation of BMSCs by activating Wnt signaling (Cui et al. 2016). However, authors have not illuminated the special molecule that works in ST2 cells. EVs derived from the bone marrow interstitial fluid of aged mice can inhibit the osteogenic differentiation of young BMSCs by decreasing the heme oxygenase-1 (Hmox1) level (Davis et al. 2017). Hmox1 is sensitive to oxidative stress and promotes osteoblastic differentiation of BMSCs (Lin et al. 2010).

EVs in Bidirectional Osteoblast-Osteoclast Communication

Bidirectional osteoblast-osteoclast communication plays crucial roles in bone remodeling. MVs from osteoblasts

Table 2. Published Studies on Biogenesis of EVs in Bone Remodeling.

EVs: Source and Kind	Methods: Isolated and Identified	EVs: Rations	Specific Cargo	Target Cells and Genes	Functions	Citations: First Author and Year
• Human BMSCs • EVs	• Differential centrifugation: 500 × *g* for 30 min, 16,500 × *g* for 20 min, filtration through a 0.22-μm filter, 120,000 × *g* for 120 min • TEM and flow cytometry (CD63)	Total protein (BCA assay)	miR-196a	Human osteoblasts (hFOB 1.19)	Promote osteogenic function and bone regeneration	Qin 2016
• Bone marrow interstitial fluid (supernatant) of young (3 to 4 mo) and aged (24 to 28 mo) mice • MVs and exosomes	• Total Exosome Isolation Reagent for serum (Life Technologies) • Immunogold labeling (CD63, CD9)	Total protein (BCA assay)	miR-183-5p	• Young BMSCs • Hmox1	Suppress osteogenic differentiation, increase cell senescence	Davis 2017
• Mouse BMSCs • MVs and exosomes	• Differential centrifugation: 300 × *g* for 10 min, 2,000 × *g* for 30 min, 10,000 × *g* for 1 h, and 100,000 × *g* for 1 h with collection of the 100,000 × *g* pellet (exosomes), 10K pellet (large vesicles, microvesicles), 100K to 10K pellet (small vesicles, exosomes), and the 100K pellet (no 10K spin, small and large vesicles, exosomes and microvesicles) • WB (CD9, CD63, and CD81) and NanoSight	EVs number (NanoSight)	miR106b-3p, miR155-5p, and miR210-5p	murine hematopoietic cell line (FDC-P1 cells)	Promote proliferation (microvesicles and exosomes > microvesicles > exosomes); reversal of DNA damage and apoptosis	Wen 2016
• Human osteoblast cell line (UAMS-32P cells) • MVs	• Differential centrifugation: 300 × *g* for 10 min, 2,000 × *g* for 10 min, further centrifugation at 16,000 × *g* at 4 °C for 60 min, centrifugation at 16,000 × *g* for another 60 min • TEM	MVs released from about 4×10^7 UAMS-32P cells	RANKL	• Osteoclast precursors (RAW264.7 cells) • RANK	Facilitate osteoclast formation	Cui 2016
• Mouse preosteoblast cell line (MC3T3-E1 cells) • Exosomes	• Exoquick reagent (System Biosciences) • TEM	Total protein (BCA assay)	miRNAs	• Bone marrow stromal cell lines (ST2 cells) • Axin1; beta-catenin (Wnt signaling pathway)	Promote osteogenic differentiation	Deng 2015
• Mouse preosteoblast cell line (MC3T3-E1 cells) • Exosomes	• Exosome extraction kit (Life Technologies) • WB (CD63) and TEM	Total protein (BCA assay)	TRIP-1	• Type I collagen	Promote mineralization	Ramachandran 2016
• Osteoclast precursors • EVs	• ExoQuick reagent (System Biosciences) • TEM and WB (CD63, gp96, and calnexin; endoplasmic reticulum proteins)	EVs Number (EXOCET Kit)	No RANK	Isolated mouse marrow hematopoietic precursors: RANKL and CSF-1 or with 1α,25(OH)2D3	Stimulate osteoclastogenesis	Huynh 2016
• Mature osteoclasts • EVs	• ExoQuick reagent (System Biosciences) • TEM and WB (CD63, gp96, and calnexin; endoplasmic reticulum proteins)	EVs Number (EXOCET Kit)	RANK	Isolated mouse marrow hematopoietic precursors: RANKL and CSF-1 or with 1α,25(OH)2D3	Inhibit osteoclastogenesis	Huynh 2016

(continued)

Table 2. (continued)

EVs: Source and Kind	Methods: Isolated and Identified	EVs: Rations	Specific Cargo	Target Cells and Genes	Functions	Citations: First Author and Year
• Osteoclasts • Exosomes	• Differential centrifugation: 300 × *g* for 10 min at 4°C, 820 × *g* for 15 min, 10,000 × *g* for 5 min at 4 °C and passage through a 0.8-mm filter, 100,000 × *g* for 2 h at 4 °C • Flow cytometry (CD63, CD9) and NanoSight	Total protein (BCA assay)	miR-214-3P	Osteoblasts	Inhibit osteoblastic bone formation	Li 2016
• Human synovial fluid from OA • Exosomes	• Total Exosome Isolation Reagent (Life Technologies) • WB (CD81, CD63, and TSG101), NanoSight, TEM and immunogold labeling of exosomes	Total protein (a Bradford assay)	miR-26a	Articular chondrocytes	Decrease anabolic genes and elevate catabolic and inflammatory genes	Xie 2015; Kolhe 2017; Yin 2017
• Mouse BMSCs • Exosomes	• Differential centrifugation: 300 × *g* for 10 min, 2,000 × *g* for 10 min, 10,000 × *g* for 30 min, and 100,000 × *g* for 70 min • Exo-Flow kits (CD63)	EXOCEP exosome quantitation kit	miR-151-5p	BMSCs	Promote osteogenic differentiation	Chen 2017

BCA, bicinchoninic acid assay; BMSC, bone marrow stem cell; EV, extracellular vesicle; MV, microvesicle; OA, osteoarthritis; RANK, receptor activator of nuclear factor κB; RANKL, RANK ligand; Hmox1, heme oxygenase-1; TRIP-1,TGF beta receptor II interacting protein 1; TEM, transmission electron microscopy; WB, Western blot.

(UAMS-32P) can be bound to osteoclasts, and this process is visualized by the MV tracing system. In this system, MVs membranes, as originated from the cell plasma membrane, are labeled by green fluorescent protein (GFP) after UAMS-32P cells are transfected by a GFP-tagged lentivector. When cocultured with these MVs, osteoclast precursors express GFP, which suggests osteoblast-osteoclast communication (Deng et al. 2015). However, osteoclasts also deliver their EVs to osteoblasts and regulate osteoblast activity (Li et al. 2016).

EVs in Osteocyte-Osteoblast Communication

Osteocytes, the terminally differentiated cells, play multifunctional roles in the regulation of bone remodeling (Plotkin and Bellido 2016). To elucidate the regulatory mechanisms of osteocytes, many studies focused on the role of osteocyte-derived EVs. Several miRNAs, such as miR-29, miR-484, and miR-221, exist in exosomes derived from mouse osteocytes (MLO-Y4 cells) (Sato et al. 2017). In dentin matrix protein 1–driven diphtheria toxin receptor transgenic mice (osteocyte-less mice), these miRNAs exhibit decreased expression in exosomes. This finding indicates that osteocytic exosomes can circulate in the body. When cocultured with osteoblasts, osteocytic exosomes colocalize with the nucleus in MC3T3-E1 cells and inhibit the osteoblastic differentiation (Qin et al. 2017).

EVs in Osteoclast-Osteoclast Communication

MVs from osteoclast progenitors and osteoclasts have different contents. RANK is detected in 1 of 32 osteoclast-derived EVs by an immunoaffinity purification assay. The expression of RANK in MVs is higher at a later stage of osteoclast differentiation. However, the EVs from osteoclast precursors do not contain RANK. EVs derived from osteoclasts inhibit osteoclast progenitor differentiation via competitive RANKL-RANK interactions (Huynh et al. 2016).

EVs in Bidirectional Immune Cell–BMSC Communication

Crosstalk between immune cells and bone cells participates actively in the bone-remodeling process. EVs derived from BMSCs act as immunomodulatory mediators in cellular communication. BMSC-derived EVs fuse to T cells and modulate their maturation, apoptosis, and proliferation (Del Fattore et al. 2015; Chen et al. 2016; Pachler et al. 2017). However, EVs from immune cells, such as DCs, are endocytosed by human BMSCs and promote their recruitment and migration (Silva et al. 2017). Exosomes from monocytes stimulate the osteogenic differentiation of BMSCs (Ekström et al. 2013).

Roles of Molecular Components of EVs in Bone Remodeling

Many kinds of bioactive molecules (miRNAs, proteins, and signaling peptides) in EVs could be delivered to target cells and modulate bone formation and resorption.

miRNAs

miRNAs have emerged as important posttranscriptional regulators in bone remodeling (Luan et al. 2017). miRNAs can be secreted to the microenvironment via EVs and play important roles in cellular communication.

miR214-3p. Osteoclasts secrete miR214-3p mainly via exosomes. Exosomal miR214-3p targets osteoblasts to inhibit

bone formation in vitro and reduce bone formation in aging ovariectomized mice (Li et al. 2016).

miR-218. miR-218 is found in osteocytic exosomes. Muscles secrete myostatin to downregulate the level of miR-218 in exosomes. These exosomes inhibit osteoblastic differentiation by targeting Wnt signaling molecules, such as TCF7 and SOST, in MC3T3-E1 cells (Qin et al. 2017). This finding suggests that miRNAs in osteocytic exosomes are a novel mechanism in muscle-bone communication.

miR-183-5p. The cargo of EVs derived from the bone marrow interstitial fluid of aged mice is different from that in young mice. The former contains high levels of the miR-183 cluster (miR-96/-182/-183), which is associated with oxidative stress and aging. These EVs can reduce proliferation and osteogenic differentiation of young BMSCs by targeting Hmox1 (Davis et al. 2017).

miR-196a. miR-196a, miR-27a, and miR-206 are highly expressed in human BMSC-EVs. Upon functional analysis, these miRNAs exhibit osteogenic effects, and miR-196a is the most influential. Moreover, cotreatment with a miR-196a inhibitor can effectively attenuate the osteogenic effects of these EVs (Qin et al. 2016).

miR-151-5p. Exosomal miR-151-5p from exogenous BMSCs can be endocytosed into endogenous BMSCs and promote osteogenic differentiation. Systemic injection of exosomes rescues osteopenia via miR151-5p delivery in vivo (Chen et al. 2017).

Other miRNAs. miRNAs in exosomes derived from BMSCs promote fracture healing (Furuta et al. 2016). Many kinds of miRNAs, such as miR-199b, miR-1192, and miR-680 (previously reported to be involved in bone remodeling), were found in bone-related EVs (Xu et al. 2014; Cui et al. 2016).

Proteins and Signaling Peptides

EphrinA2. Osteoblasts recognize the osteoclast-derived exosomes via interactions between EphrinA2 and EphA2. EphrinA2 is located on the surface of exosomes and can bind EphA2, the receptor on osteoblasts. When EphrinA2 or EphA2 is knocked down, miR-214-3p in osteoclasts-derived exosomes cannot be endocytosed by osteoblasts (Sun et al. 2016).

Sema4D. Sema4D, a previously identified osteoclast membrane protein, is secreted in osteoclast-derived exosomes (Li et al. 2016). Sema4D can target its receptor (Plexin B) expressed in osteoblasts. The Sema4D–Plexin B interaction promotes the release of EV cargo and accelerates bone formation.

RANKL. RANKL can be encapsulated into MVs in human osteoblast cell lines. Prior studies demonstrated that only with the assistance of osteoprotegerin can RANKL be packaged into secretory lysosomes (Aoki et al. 2010). When treated with parathyroid hormone, osteoblasts secrete more MVs containing RANKL, which stimulate osteoclast differentiation in the coculture system (Deng et al. 2015).

RANK. MVs from osteoclasts contain RANK. Moreover, the proportion of RANK-containing MVs increases in the late stage of osteoclast differentiation. These EVs inhibit osteoclast differentiation via RANK-RANKL interactions (Huynh et al. 2016). This finding indicates that osteoclasts could modulate the osteoclast differentiation via RANK delivery by EVs.

TRIP-1. Transforming growth factor beta receptor II interacting protein 1 (TRIP-1), a regulator of osteoblast function, is loaded into exosomes from osteoblasts. TRIP-1 in exosomes can bind to type I collagen and promote mineralization of the extracellular matrix (Ramachandran et al. 2016).

Cell Migration/Recruitment-Related Cytokines. OPN, MMP-9, and MCP-1 are found in DC-derived EVs. OPN is located inside of EVs. The active form of MMP-9 is also present in EVs, while the pro-MMP-9 form is on the membrane or transmembrane. These cytokines are transported by DC-EVs and modulate the migration and recruitment of BMSCs (Silva et al. 2017).

Other Proteins and Signaling Peptides

MVs derived from growth plate cartilage contain growth factors and signaling proteins, including BMP1-7, BSP, vascular endothelial growth factors, OCN, OPN, and ON, as determined by Western blotting and enzyme-linked immunosorbent assay (ELISA) (Nahar et al. 2008). The exosomes can increase ALP activity in cultured chondrocytes. Many extracellular matrix proteins, such as collagen, fibronectin, and periostin, are also found in osteoblast-MVs (Xiao et al. 2007).

Potential Role of EVs as Biomarkers in Bone-Related Diseases

Bone formation and resorption are the basis of bone metabolism balance. When this balance is disturbed, bone-related diseases occur. Multiple myeloma (MM), a malignant osteolytic bone disease, is characterized by excessive osteoclastic activity and deficient osteoblastic activity. Communication between bone marrow–derived cells and MM cells plays an important role in bone resorption and tumor progression (Röllig et al. 2015). Exosomes derived from MM cells promote osteoclast precursor recruitment and differentiation (Raimondi et al. 2015). In addition, EVs derived from MM-BMSCs accelerate the proliferation of MM cells, whereas EVs from normal BMSCs suppress the proliferation and angiogenesis of MM cells (Ohyashiki et al. 2018). The fact that serum EVs express the high level of CD31 (a MM-related antigen) in patients with MM demonstrates that the circulating exosomal cargo is an independent diagnostic and prognostic biomarker in MM (Quarona et al. 2015).

Osteoporosis is a common and serious disease characterized by reduced bone mass and increased risk of bone fracture. There is elevated serum exosomal miR-214-3p in elderly patients with fractures. miR-214-3p in exosomes prevents osteoblastic differentiation and bone formation (Li et al. 2016). miR-31 is also increased in the plasma of elderly individuals or

osteoporosis patients. Microvesicular miR-31 is taken up by mesenchymal stem cells and inhibits osteogenic differentiation by decreasing the levels of frizzled-3 (Weilner et al. 2016). In 1 study, miR-21-5p was downregulated in the serum of postmenopausal women with low bone mass as compared with the controls (Yavropoulou et al. 2017). Further studies, especially large-scale prospective studies, are required to determine whether these exosomal miRNAs can serve as screening biomarkers for osteoporosis.

Osteoarthritis (OA) is characterized by serious cartilage degeneration in articulating joints and is highly prevalent among women (Kolhe et al. 2017). Early diagnosis of OA is difficult, and treatment options for OA are limited. Synovial fluid (SF) has direct contact with the articular cartilage, synovium, and ligament in the joint microenvironment and is significantly involved in the pathogenesis of OA. Many recent studies focused on the role of EVs derived from SF or synoviocytes in the pathology of OA and on potential biomarkers for the diagnosis of OA (Xie et al. 2015; Miyaki and Lotz 2018). The sizes and concentrations of exosomes derived from SF are similar between osteoarthritic and nonosteoarthritic patients. However, when exosomes from patients with OA are endocytosed by articular chondrocytes, the expression of extracellular matrix synthesis genes (aggrecan, COL-II) decreases, and inflammatory gene (interleukin 6) expression increases (Kolhe et al. 2017). Immune cells such as DCs also secrete exosomal miRNAs to modulate inflammation in rheumatoid arthritis (Withrow et al. 2016). Moreover, exosomal miRNA profiles reveal sex specificity between female and male patients. There are many kinds of estrogen-responsive miRNAs in female OA exosomes, such as miR-181d-3p, miR-185-5p, and miR-7107-5p, which indicates that these exosomal miRNAs can act as sex-specific diagnostic markers of OA (Kolhe et al. 2017). The levels of miRNAs such as miR-26a and miR-26b are negatively correlated with OA severity, suggesting that monitoring miRNAs may serve as a biomarker for tracking OA progression (Yin et al. 2017).

Role of EVs in Tooth Development and Regeneration

In addition to bone-related cells, dental cells, such as dental pulp cells, periodontal ligament cells, and mesenchymal cells, secrete EVs (Pivoraitė et al. 2015; Huang et al. 2016; Jiang et al. 2017). Tooth development is tightly regulated by the crosstalk between epithelium-mesenchyme and various growth factors. Exosomes play vital roles in epithelium-mesenchyme crosstalk during tooth development. Epithelial exosomes promote odontoblastic differentiation and mineralization in mesenchymal cells, while exosomes derived from mesenchymal cells induce epithelium cells to produce basement membrane components, amelogenenin, and ameloblastin (Jiang et al. 2017).

Dental-related EVs also play an important role in tooth regeneration. Exosomes derived from human dental pulp stem cells (hDPSCs) could promote odontogenesis (Huang et al. 2016). Furthermore, the exosomes could promote the regeneration of dentin-pulp tissue, when applied to a tooth root slice regeneration model (combination of collagen membranes, hDPSCs, and exosomes). Glial cells in the peripheral nerve system, such as Schwann cells, have key immunomodulatory functions in dentin repair (Couve et al. 2017). One study recently revealed that Schwann cells secrete EVs to maintain the proliferation and multipotency of hDPSCs, which provides new insights into the regulatory mechanisms of the nervous system in tooth regeneration (Li et al. 2017). In addition, dental exosomes provide protection against pathologic processes, such as inflammation and oxidative stress (Jarmalavičiūtė et al. 2015; Pivoraitė et al. 2015).

Promising Prospects and Challenges of EV Application

The discovery of EVs provides new insights into intercellular communication in bone remodeling. Cargo specificity and ease of harvest are 2 prominent advantages in the diagonstic application of EVs as promising biomarkers (Quarona et al. 2015; Yin et al. 2017). EVs can also be used as therapeutic agents by themselves in diseases (Huang et al. 2016; Kamerkar et al. 2017). When osteoporosis occurs, strategies to accelerate fracture healing are important. Exosomes derived from BMSCs can enhance fracture healing via miRNA delivery (miR-21, miR-4532, and miR-125b-5p; Furuta et al. 2016). Because of EVs' nanosize, high biocompatibility, and specific binding sites on the membrane, it is an attractive option to develop EVs as targeted delivery systems in skeletal diseases. For instance, EphrinA2, located on the surface of exosomes, can bind its receptor specifically on osteoblasts (Sun et al. 2016). Loading therapeutic drugs such as parathyroid hormone into the EphrinA2-tagged exosomes is an efficient way to regulate osteoblast activity in osteoporosis. EVs could also stand out in bone regeneration, due to their low immunogenicity profile as compared with BMSCs. BMSC-derived EVs could enhance bone formation when mixed with decalcified bone matrix scaffolds in bone regeneration (Xie et al. 2017).

However, a systematic understanding of how to preserve the best function of EVs in bone remodeling must be developed. First, isolation of high-purity exosomes provides more exact diagnostics and efficiency of therapies. Many isolation protocols are used to study EVs, such as differential centrifugation, density-gradient centrifugation, and ultrafiltration. However, these protocols cannot maximize the purity of EVs (Coumans et al. 2017). Although some modifications are efficient strategies for EV isolation, such as the biotin modification of the EV membrane, standard guidelines are urgently needed (Zhang et al. 2017). In addition, exosomes are eliminated in the liver within a short time (Morishita et al. 2015), which indicates that some EVs are eliminated before they take effect. Therefore, improvement of targeted delivery and efficiency of EVs is a major issue in EV-based applications. Modification of the osteoblast-EVs with anti-bone-resorptive drugs would improve the treatment efficiency in osteoporosis. When EVs are loaded with

chemotherapeutic agents, they would focus on the tumor and protect the adjacent tissues.

Conclusion

In summary, EVs are carriers for biomolecules to send intercellular messages in the bone-remodeling microenvironment. EVs exert positive or negative roles in the bone remodeling during physiologic and pathologic conditions. The contents of EVs act as the potential biomarkers in MM, osteoporosis, and OA. Meanwhile, EVs exert beneficial effects in bone regeneration and targeted treatment of bone diseases.

Author Contributions

M. Liu, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; Y. Sun, contributed to conception, design, and data acquisition, drafted and critically revised the manuscript; Q. Zhang contributed to conception, design, data analysis, and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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