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Chapter

Potential Therapeutic Applications of Exosomes in Bone Regenerative Medicine

Jiazhao Yang, Wanbo Zhu, Jinsen Lu, Kai Xie, Shiyuan Fang and Lixin Kan

Abstract

The ability of bone regeneration is relatively robust, which is crucial for fracture healing, but delayed healing and nonunion are still common problems in clinical practice. Fortunately, exciting results have been achieved for regenerative medicine in recent years, especially in the area of stem cell-based treatment, but all these cell-based approaches face challenging problems, including immune rejection. For this reason, exosomes, stem cell-derived small vesicles of endocytic origin, have attracted the attention of many investigators in the field of bone regeneration. One of the attractive features of exosomes is that they are small and can travel between cells and deliver bioactive products, including miRNA, mRNA, proteins, and various other factors, to promote bone regeneration, with undetectable immune rejection. In this chapter, we intend to briefly update the recent progressions, and discuss the potential challenges in the target areas. Hopefully, our discussion would be helpful not only for the clinicians and the researchers in the specific disciplines but also for the general audiences as well.

Keywords: exosome, stem cell, fracture healing, osteogenesis, bone regeneration

1. Introduction

Fractures are common traumatic injuries during the entire human history. Both traditional and modern medicine have kept on exploring and researching on many potential treatments. Despite these efforts and relatively robust regenerative capacity of bone, currently, there are still about 5–10% fracture patients face delayed fracture healing and even nonunion, which has a great negative impact on the quality of life of patients as well as their families [1]. Surgical intervention with autologous bone graft seems to be the preferred method for such complication, but the secondary trauma and the limited resources of grafting bone make this approach still unsatisfactory [2, 3]. Other methods, including active substance injection and bone marrow transplantation, are also used clinically but they face their own challenges, including the effectiveness, safety and immune rejection [4, 5]. Therefore, how to promote fracture healing efficiently and safely is still the major focus of recent research in regenerative medicine for bone.

Normal bone regeneration is a complex but well-orchestrated physiological process that includes the initiation of ossification, osteoinduction, and osteogenesis

[6–9]. Specifically, when bone injury occurs, a series of signaling pathways is activated, which, in turn, leads to angiogenesis and other downstream events, and these together establish a favorable microenvironment, which set the stage for stem cell based fracture healing/regeneration [10]. Within this microenvironment, abundant blood vessels accelerate the metabolism while bringing a large number of multipotential stem cells [11, 12]. On the other hand, the mononuclear phagocyte system from the blood differentiates into osteoclasts in the newly established microenvironment, and the bone resorption, in turn, specifically stimulates the bone re-modeling process [13, 14]. During the stereotyped osteogenesis process, stem cells proliferate and differentiate into osteoblasts and migrate to areas of bone defects and bone resorption, secreting collagen matrices [7, 15–17], and then immature osteoblasts produce bone matrix containing calcium and phosphate to promote mineralization [18]. Of note, new blood vessels in the fracture microenvironment can also bring essential nutrients and mineral salt for fracture healing, improving the efficiency of osteogenic differentiation and bone regeneration [19].

Embryonic stem cell transplantation was considered as a potential promising treatment for tissue repair; however, due to the limitation of donor cells and biosafety issues, its clinical application has not been widely accepted [20–23]. Recently, it has been recognized that adult bone marrow-derived mesenchymal stem cells (BMSCs) might be a better alternative, and moreover, researchers found that BMSCs play an important role in promoting tissue regeneration through paracrine signaling [24, 25], in addition to directly differentiation into bony tissue. This paracrine effect, mediated by signaling molecules, transcription factors, and other proteins, regulates a series of signaling pathways involved in bone regeneration.

Interestingly, extracellular vesicle derived from stem cells under specific stimulation can carry specific substances produced by paracrine secretion and transmit to target organs/cells to act as an intercellular communicator [26, 27]. Among all the extracellular vesicles, the particles with the diameter around 40–100 nm are commonly called exosomes. Further study found, that in addition to stem cells, many other cells, such as osteoblasts, can also produce exosomes [28]. The key unanswered question is: could these different cell-derived exosomes promote bone regeneration and accelerate fracture healing? This chapter will focus on this important question.

2. A brief overview of exosome

In 1983, Harding found a lysosomal-like vesicle in reticulocytes of rats. It was found that transferrin was internalized by this vesicle and its receptors also recycled back to the plasma membrane through endocytosis [29]. In 1987, such vesicle-like structures were also found in the culture medium of sheep red blood cells cultured in vitro by Johnstone, and the vesicles were later named as exosomes [30]. It is now accepted that the extracellular vesicles secreted by cells could be generally classified as microvesicles, apoptotic bodies, and exosomes, on the basis of the size, cellular origin, content, and biological function [31, 32]. Currently, the exosomes are extensively studied. Exosomes, normally 40–100 nm in diameter, have been defined as a type of extracellular vesicles with unique biological features and morphology (flat or cup-shape under electron microscope) [33, 34] (**Figure 1**). The formation of exosome is essentially the encapsulation of bioactive substances, including proteins and nucleic acids, into multivesicular bodies with the help of endosomal sorting complex in the cells [35, 36]. The newly formed exosomes inside the cell are transported and fused with the plasma membrane and eventually released into the extracellular matrix [37, 38].

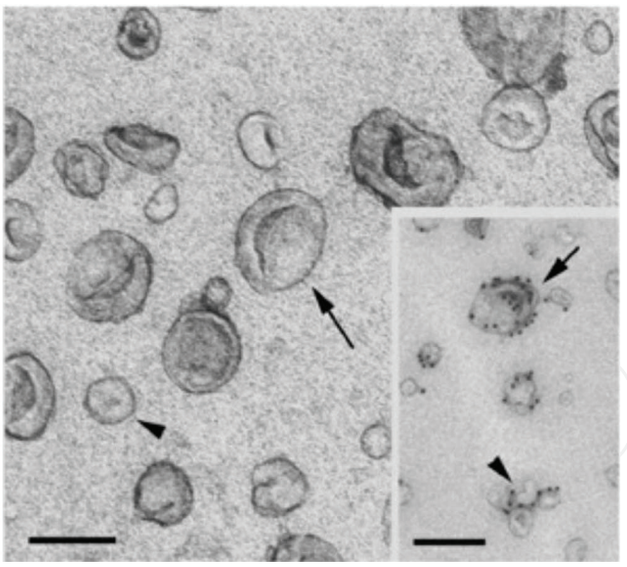

Figure 1.
Electron-microscopic observation of whole-mounted exosomes purified from mouse dendritic cells. Arrows indicate exosomes, arrowheads point to smaller nonexosomal vesicles. Scale bar = 100 nm. (Quote from Théry et al. [33].)

It is now known that numerous different type of cells, including dendritic cells, mast cells, lymphocytes, neurons, and endothelial cells secrete exosomes [39–43], which are found in blood, amniotic fluid, urine, malignant ascites, and other body fluids such as bile [44–47]. The key features of exosomes as intercellular communicators is due to the fact that they are able to selectively carry the contents of the parent cells and act on target cells [31, 38]. In 2007, Valadi found that exosomes contain RNA, which indicated exosomes might regulate genetic information flow [48]. In recent years, many studies have found that a variety of cell-derived exosomes contain mRNA and miRNA and play an important role in cell-to-cell signaling [48–50]. Therefore, the transport of RNA and active proteins through exosomes provides a novel pathway for activating target cell and initiating and propagating downstream signaling pathways. For example, in 2012, Cantaluppi discovered that microvesicles from epithelial progenitor-derived cell initiated renal-regeneration procedures by carrying miRNAs and acting on target cells, reversing focal ischemic lesions [51].

The regenerative effects of exosomes have been validated in other tissues and organs, including the heart, lungs, kidneys, and brain [52–54]. For example, in a mouse model of myocardial infarction, treatment of exosomes can improve cardiac epicardial remodeling and increase left ventricular ejection fraction [55]. In hypoxic-induced pulmonary hypertension, exosome treatment inhibits disease progression and protects the lungs from hypertension [56]. In addition, exosome treatment can improve renal function in a mouse model of acute kidney injury [57]. These studies indicate that exosomes have the capacity to promote tissue regeneration, which provides a basis for their potential application in bone regeneration [58].

3. Exosomes in bone regeneration

3.1 The exosomes derived from different cells promote bone regeneration

The mechanism of stem cells in the treatment of diseases has not been fully elucidated; however, it is now commonly accepted that there are two recognized mechanisms: differentiation and paracrine. In fact, it is becoming clearer that

paracrine mechanism could be a more important mechanism; therefore, exosomes, as important mediators in paracrine mechanism, have attracted researchers.

Embryonic stem cells are considered to be the ideal materials for regenerative medicine because of their ability of pluripotent differentiation. But later study found that bone marrow mesenchymal stem cell (BMSC) could be a better alternative, i.e., BMSCs are self-renewing mesodermal pluripotent stem cells that can differentiate into osteoblasts, fat cells, nerve cells, and myoblasts [24, 59]. Recent study also found that BMSCs' roles in inducing angiogenesis, regulating inflammation, inhibiting apoptosis, and regulating osteogenesis differentiation make them desirable for bone regeneration applications [59].

Similarly, the adipose-derived stem cells (ADSCs) can also be osteogenic differentiated to promote bone regeneration, when they have been applied to the bone defects using a composite biological scaffold [60]. In addition, endothelial progenitor cells (EPCs) can differentiate into vascular endothelial cells to generate blood vessels, and promote MSCs osteogenesis in a specific microenvironment [61, 62]. Also, differentiated cells, such as osteoblasts and osteoclasts, also have the ability to promote bone regeneration [15, 26].

More importantly, numerous studies suggest that the above-mentioned cell-derived exosomes all have a certain ability to promote bone regeneration, through regulating bone regeneration procedures such as angiogenesis, osteogenic differentiation, and bone mineral deposition. However, the capacities and regeneration mechanisms of exosomes from different derived cells are somewhat inconsistent, likely due to their different contents.

3.2 Genetic materials carried by exosomes regulate bone regeneration

It was reported that stem cell-derived exosomes can carry genetic materials such as miRNA and mRNA, and share these genetic information between mature bone cells and stem/progenitor cells, which is an important way to promote bone regeneration [63]. MicroRNAs (miRNAs) are thought to be important posttranscriptional regulators of osteoblast-associated osteogenesis and bone remodeling, enabling a range of bone regenerative responses [64, 65]. Interestingly, miRNAs, inside the lipid membrane of exosomes, can avoid the decomposition of immune system; therefore, they exert their effects more efficiently [66].

Many researchers reported that some stem cell-derived exosomal miRNAs have the ability to activate osteogenic differentiation and angiogenesis of target cells and promote bone formation. For example, Xu first found that exosomal miRNA is a regulator of osteoblast differentiation [67]. Similarly, a series of miRNAs, such as let-7a, which could enhance the osteogenic differentiation of stem cells and promote bone regeneration, are significantly upregulated [68]. These data all demonstrated that stem cell-derived exosomes could promote bone regeneration by carrying specific miRNAs (**Table 1**).

Furthermore, many recent studies focus on MSCs-derived exosomes (BMSC-Exo) for bone regeneration. For examples, in CD9−/− mice, BMSC-Exo isolated from culture medium can accelerate fracture healing compared with the control group [69]. In vitro analysis of the exosomes revealed that miR-21, miR-4332 and other osteogenic differentiation-related miRNAs are highly expressed compared to other cell-derived exosomes. Interestingly, mononuclear cell chemotactic protein MCP-1/-3 and stromal cell-derived factor SDF-1, were lower in BMSC-Exo than in the control group [70, 71]. This might suggest that differential distribution of osteogenic differentiation and angiogenesis-related miRNAs in BMSC-Exo. In another study, BMSC-Exo group showed a significant increase in bone formation and repair rate in the model of mouse skull repair, compared with the control group. Similarly, in vitro experiments,

miRNA	Derived cells	Express level	Target cells	In vivo evaluation	In vitro evaluation	Involved pathway
Let-7a [67]	BMSCs	Upregulated	MSCs	Promote bone formation	Promote osteogenesis and suppress adipogenesis [68]	AXIN2 HMGA2
miR-218 [67]	BMSCs	Upregulated	SMSCs	None	Inhibit osteogenic differentiation	None
miR-203 [67]	BMSCs	Upregulated	BMSCs	None	Promote osteoblastic differentiation	None
miR-196a [59]	BMSCs	Upregulated	BMSCs	Stimulate bone formation	Positively regulated osteogenic genes and osteoblastic differentiation but did not inhibit proliferation	None
miR-27a [59]	BMSCs	Upregulated	MSCs	Overexpression promoted osteogenic differentiation	None	PPARγ
miR-206 [59]	BMSCs	Upregulated	None	None	None	None
miR-21 [69]	BMSCs	Upregulated	BMSCs/ MSCs	Accelerate fracture healing	Promote osteogenic differentiation	PI3K/AKT
miR-125b [69]	BMSCs	Upregulated	BMSCs	None	Suppresses the proliferation and osteogenic differentiation of BMSCs	None
miR-10b [72]	BMSCs	Upregulated	MSCs	None	Promote the migration of MSCs	None
miR-221 [72]	BMSCs/ MSCs	Downregulated	MSCs	Anti-miR-221 enhances bone healing	Downregulation of miR-221 triggers osteogenic differentiation	None
miR-155 [67]	BMSCs	Downregulated	None	None	None	None
miR-31 [72]	MSCs	Downregulated	BMSCs	Inhibition of miR-31 in MSCs increased bone volume and bone mineral density	Inhibit the osteogenic differentiation of MSCs	Wnt
miR-144 [72]	MSCs	Downregulated	MSCs	None	Inhibit the osteogenic differentiation of MSCs	None

Table 1.
Summary of Exosomal miRNAs and their potential effects on bone metabolism.

BMSC-Exo was showed to activate osteogenic differentiation, increase osteoblast activity, and promote bone formation without inhibiting stem cell proliferation [59].

Further study found that the ability of exosomes to promote bone formation is different even when the parent cells are in different differentiation stages. For example, in vitro experiments demonstrated that the human mesenchymal stem cell-derived exosomes (hMSCs-Exo) from the late differential stage have the strongest osteogenic differentiation ability [67, 72]. Consistently, MiR-31, miR-221, and miR-144 that inhibit osteogenic differentiation have significant decreased levels in late differential stage of hMSCs-Exo, while miR-21, miR-10b, and other miRNAs that contribute to osteogenesis is significantly upregulated [73–76]. It should be noted that the exosome miRNA's ability to regulate cell function could be context dependent, especially in the present of inhibitory miRNAs [67, 77]. Therefore, to promote bone regeneration using stem cell-derived exosomes, silencing inhibitory miRNAs may be a problem to be solved.

In addition, some miRNAs carried by other cell-derived exosomes also have the ability to promote bone regeneration. For example, the mineralization-related miR-503-3p is highly expressed in the miRNAs carried by osteoblast-derived exosomes. Interestingly, miR-503-3p also inhibits osteoclast differentiation by mediating RANK expression [78]. Osteoblast-derived exosomes and pre-osteoblasts-derived cells can also carry miRNAs such as let-7a and miR-96a, which have been previously confirmed to be involved in bone remodeling [79]. Similarly, the miR-27a-3p carried by myogenic cell-derived exosomes can also enhance osteogenic differentiation of pre-osteoblasts [80]. In contrast, osteoclast-derived exosomes can carry miRNAs such as miR-214 that inhibit osteogenic differentiation of osteoblasts [81]. Interestingly, in vitro experiments have found that human adipose stem cell-derived exosomes (ASCs-Exo) can increase the osteogenic capacity of target cells by upregulating the mRNA expression of osteogenesis-related genes RUNX2, ALP, and COL1A1, and promote bone formation [82]. In addition, the mRNA of RAB13, an osteoclastic membrane trafficking protein required for bone resorption, is also overrepresented in osteoblast-derived exosome [49].

Overall, cell-derived exosomal miRNAs and mRNAs likely play important roles in bone regeneration, through promoting osteogenic differentiation, angiogenesis and other processes. However, it is unclear whether protein factors are eventually needed to mediate their final effects.

3.3 Key protein factors carried by exosomes regulate bone regeneration

Key factors in stem cell-derived exosomes are known to mediate a series of conserved signaling pathways.

RUNX2 is an important transcription factor that can regulate osteogenesis differentiation, through promoting the differentiation of pluripotent stem cells into osteoblasts and inhibit osteoblast maturation [83]. Consistently, in vivo experiment found that human induced pluripotent mesenchymal stem cell-derived exosomes (hiPS-MSC-Exo) stimulated osteogenic differentiation, promoted angiogenesis, and improved fracture healing rate in animals with the upregulated transcription factors such as RUNX2 [84]. It was also reported that cell derived exosomal miRNAs are critical for upregulation of RUNX2 [85, 86]. Interestingly, RUNX2 directly represses miR-31 expression, which significantly inhibits expression of the osteogenic transcription factors OPN, BSP, Osterix (OSX), and OCN [87].

PI3K-AKT signaling pathway is thought to play an important role in exosomes-mediated bone regeneration because it stimulates osteogenic differentiation and promotes osteogenesis [88, 89]. Consistently, Shabbir et al. found that BMSCs-Exo activates multiple signaling pathways including Akt, Erk1/2, and STAT3 to induce angiogenic responses in fibroblasts [90]. In vitro experiment also found that

hiPS-MSC-Exo downregulates inhibitory factor (GSK3β and PTEN) by upregulating PI3K-AKT target genes PDGFA and FGFR1 [91], and activation of PI3K-AKT cascade induces stem cell proliferation and differentiation into osteoblasts, and enhances ALP expression and calcium salt deposition, promoting bone regeneration. In the context of long-term nonunion of the femoral neck fracture or intertrochanteric fracture induces femoral head necrosis, Liu et al. found that iPS-MSCs-Exo activates the PI3K/Akt signaling pathway to increase angiogenesis and reduce bone loss [94].

miRNAs are also important molecules that regulate the PI3K-AKT signaling pathway. For example, miR-21, highly expressed in BMSCs-Exo, is one of the major regulators in stem cell-derived exosomes, which promotes osteogenic differentiation not only by inhibiting SOX2 [92], but also regulating the PI3K-AKT-GSK3β signaling pathway, which, in turn, activates the transcription of RUNX2, and stimulate osteogenic differentiation [93].

Wnt pathway is an important signaling pathway related to bone repair. In this regard, ASCs-Exo pretreated with TNF-α could upregulate Wnt3 expression in stem cells and promote bone regeneration [95, 96]. Zhang et al. also found that human umbilical cord stem cell-derived exosomes induce Wnt4-mediated β-catenin nuclear transport, and induce endothelial cell proliferation, differentiation, and neovascularization [97]. Similarly, BMSCs-Exo also activates the Wnt3a-β-catenin pathway and induces angiogenic capacity of fibroblast [98].

RANKL-RANK signaling is known to be responsible for homeostasis of bone metabolism, which is determined by a dynamic balance between osteoclasts and osteoblast [99]. Interestingly, Nuclear factor kappaB ligand (RANKL) can be encapsulated into osteoblast exosomes, while osteoclast exosomes are enriched with RANK [100]. When RANKL binds to RANK in pre-osteoclasts, TNF receptor-related factors (TRAF) 2, 3, 5, and 6 are recruited, leading to activation of multiple signaling pathways including MAPK and NF-κB, promoting osteoclast differentiation and bone resorption [101]. Moreover, level of RANK-containing exosomes increases in the late stage of osteoclast differentiation, which negatively feedbacks on RANKL-RANK signaling to inhibit osteoclast differentiation [99]. Therefore, RANKL-RANK loop contributes to the homeostasis of bone metabolism and bone regeneration.

Other proteins and cytokines in the exosomes are also involved in promoting bone regeneration process. For example, Martins et al. found that hBMSCs-Exo induced BMP2 upregulation, and BMP2 in turn, promoted stem cell osteogenic differentiation and osteogenesis by cascade activation of transcription factor OSX instead of RUNX2 [65]. Similarly, SPE1 (secreted phosphoprotein 1), integrin-binding sialoprotein and bone gland protein BGLAP (bone g-carboxyglutamate (gla) protein) were also upregulated, which facilitated bone mineralization and other bone regeneration processes. MSCs-Exo is also known to induce high expression of BMP9, transforming growth factor β1 (TGFβ1), vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF) [102]. BMP9 is considered to be an osteogenic factor stronger than BMP2. TGFβ1 and PDGF are known to play roles both in osteogenic differentiation and angiogenesis [103–105]. Qi et al. also found that hiPSC-MSC-Exo induced high expression of osteopontin, osteocalcin, and type I collagen (COL1), and enhanced bone mineralization [84, 106]. Meanwhile, high expression of phosphorylated protein and bone matrix acidic protein (DMP1) was found in the extracellular matrix (ECM) containing MSCs-Exo, suggesting MSCs-Exo promotes calcium phosphate recruitment and bone mineralization [107].

In addition, exosomes from osteoblast carry transforming growth factor beta receptor II interacting protein1 (TRIP-1), a regulator of osteoblast function. TRIP-1 from the exosomes can bind to type I collagen and promote its mineralized extracellular matrix, therefore bone mineralizing [108]. Sema4D is an osteoclast membrane protein that can be carried in exosomes derived from osteoclasts and acts on the

receptor Plexin B on osteoblasts [81]. The Sema4D-Plexin B interaction promotes the release the content of exosomes and accelerates bone formation.

It is worthy to mention that some proteins, though are highly expressed in stem cell-derived exosomes and have the potential for bone regeneration, do not seem to play important roles in exosomes mediated osteogenic or chondrogenic differentiation in different contexts. For example, heat-shock protein 70 (HSP70), which can be used as a marker of BMSCs-Exo, is downregulated in human MSC-Exos and negatively affects osteogenic and chondrogenic differentiation. Similarly, down-regulation of heat shock protein B8 (HSPB8) can reduce the formation of dental pulp stem cells, and osteogenic differentiation ability [109-111]. Overall, the specific biological mechanisms of some functional proteins to promote fracture healing are not fully understood, and further detailed researches will be needed.

4. Clinical therapeutic applications and limitations

Many studies have shown that stem-derived exosomes in vitro and in vivo activate a series of bone regeneration programs through their selective bioactive substances, which are mainly through osteogenic differentiation, angiogenesis, and bone mineralization. In these applications, the high extracellular matrix binding affinity of stem cell-derived exosomes is a big plus for their clinical application. Recently, some scholars have found that human adipose-derived stem cell-derived exosomes promote fracture healing in animals by binding to polylactic acid-glycolic acid scaffolds [82]. At the same time, the immunomodulatory and anti-inflammatory properties of stem cell-derived exosomes have also attracted the attention of researchers, which could be the potential biological mechanisms for clinical treatment to promote bone regeneration [112, 113].

However, so far there are few examples of clinical trials using exosomes as clinical treatments. At present, exosome clinical application has only been reported in the fields of treatment of chronic kidney disease, type 1 diabetes mellitus (clinical trialNCT02138331), and skin damage (clinical trial NCT02565264) [114]. In the field of bone regeneration, to our best knowledge, there is not any clinical trial, either ongoing or finished. The main reasons for this delay could be logistic, since the separation, acquisition, purification, and identification of exosomes are still in the laboratory stage, and large scale manufacture is still a major practical challenge. In addition, the healing of the fracture will take several months, and how to make the exosomes available constantly in the fracture site for such a long time is also a problem.

Cell culture: The acquisition of a large number of exosomes requires a large number of cells [115]. However, large scale stem cells culture may alter the cell phenotype [116]. Existing cell culture techniques such as bioreactors have expanded the surface area of cell growth, but it is still difficult to perfectly control the conditions of cell growth [117]. As mentioned above, exosomes from different stages of derived cells have different bone regeneration capabilities. However, there are still limitations on how to obtain batch production from the specific stage of the cells.

Purification: Ultracentrifugation and ultrafiltration can be used to obtain purified exosomes in the laboratory, but this technology is difficult to apply on a large scale [118]. The nonspecific precipitation method using polyethyleneglycol (PEG) can solve this problem well, but PEG needs to be removed again in the product, which is technical challenging [119]. The tangential-flow filtration technology based on cell size separation is currently considered promising; however, it is expensive to use and does not separate some biological materials such as DNA [118, 120].

Identification and quality control: Current laboratory identification and quality control methods include direct observation under electron microscopy and biomarkers observation, but none of them can be scaled up easily. The identification and quality control using immunomagnetic capture of exosomal biomarkers

through microfluidic technology can speed up the identification process, but it also has a long way to go before this method can be commonly accepted [118, 121].

In summary, the existing technology still has great challenges for large-scale acquisition of purified exosomes.

5. Existing disputes and problems

Whether promoting bone regeneration will indirectly lead to tumor production is a controversy that needs to be tested seriously. In fact, there are some studies have shown that exosomes can promote tumor growth and malignant transformation or inhibit tumor survival [122, 123]. For example, Qi et al. found that BMSCs-Exo can induce osteosarcoma growth by activating the Hedgehog signaling pathway [124]. BMSCs-Exo can induce drug resistance even on the basis of promoting the proliferation and differentiation of myeloma cells and the survival of migration [125, 126]. How to limit the potential tumor-promoting ability of stem cell-derived exosomes is a problem that must be solved before clinical application. However, miR-340 carried by early BMSCs-Exo can inhibit the angiogenic ability of myeloma thus significantly limiting tumor growth [127].

In clinical applications, while the short term activity of pro-osteogenic differentiation in vitro or promotion of bone regeneration is observed by exosomes treatment, the long-term activity that affects the quality of fracture healing or osteophyte formation is unknown. It is also unclear how to stop the biological effects of exosomes when the satisfactory therapeutic effect is achieved. To clarify these issues, at present, it is urgently needed to test exosomes in animal model before we can move on to clinical study.

6. Conclusion

In summary, exosomes with their carried bioactive contents have a capacity to promote bone regeneration through osteogenic differentiation, angiogenesis, and bone mineralization (**Figure 2**). Hence, exosomes are identified as potential

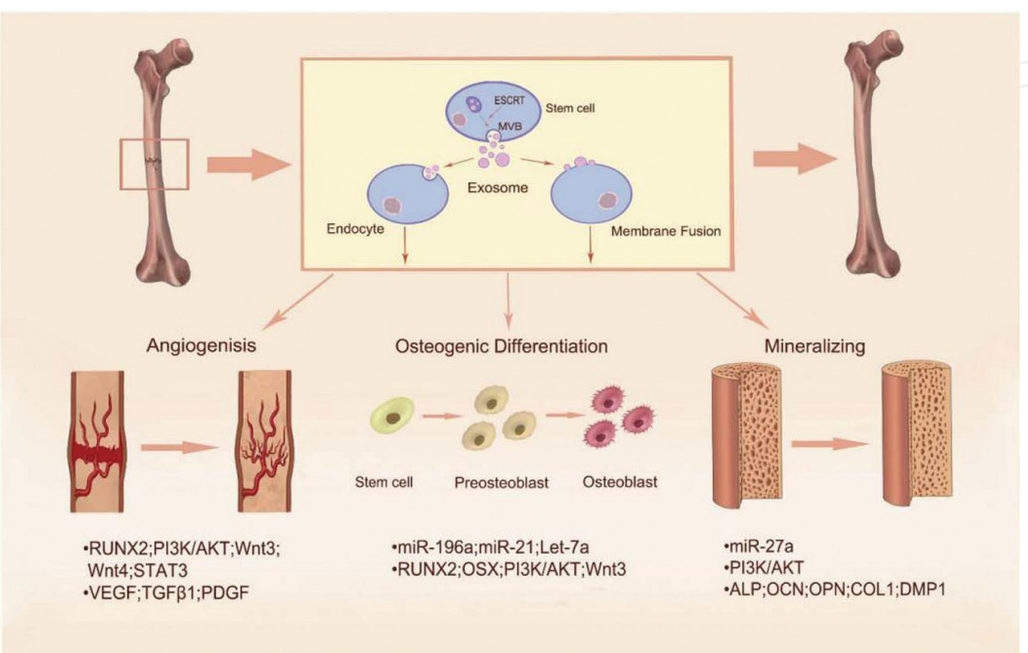

Figure 2.
Main biological mechanism of therapeutic application exosomes in bone regenerative medicine.

new "acellular" therapeutic application in bone regenerative medicine. However, clinical application of exosomes still faces controversies and challenges, and further researches are needed to elucidate the signaling pathway, molecular mechanism, and long-term clinical effect.

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Conflict of interest

The authors declare no competing interests.

Author details

Jiazhao Yang[1], Wanbo Zhu[1], Jinsen Lu[1], Kai Xie[1], Shiyuan Fang[1] and Lixin Kan[2*]

1 Department of Orthopedics, The First Hospital of University of Science and Technology of China, Hefei, Anhui, China

2 Department of Neurology, Northwestern University, Chicago, USA

*Address all correspondence to: l-kan@northwestern.edu

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