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REVIEW ARTICLE

Mesenchymal Stem Cells-Derived Exosomes: A Possible Therapeutic Strategy for Osteoporosis

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Abstract: Osteoporosis is a common age-related disorder characterized by low bone mass and deterioration in bone microarchitecture, leading to increased skeletal fragility and fracture risk. The pathophysiology of osteoporosis is multifactorial. It is related to the imbalance between osteoblasts and osteoclasts; reduced bone mass and increased adipogenesis in the bone marrow. Moreover, angiogenesis, inflammatory process and miRNAs have shown effects in the formation of osteoporosis. In the recent years, mesenchymal stem cells (MSCs) have been regarded as an excellent choice for cell-based tissue engineering therapy of osteoporosis. Growing evidence showed that paracrine effect has been considered as the predominant mechanism for the role of MSCs in tissue repair. Recently, many studies have proposed that MSCs-derived exosomes are effective for a variety of diseases like cancer, cardiovascular diseases, etc. However, whether the MSCs-derived exosomes could serve as a novel therapeutic tool for osteoporosis has not clearly described. In this review, we summarize the MSCs-derived exosomes and the relationship with osteogenesis, osteoclast differentiation, angiogenesis, immune processes and miRNAs. Finally, we suggest that MSCs-derived exosomes might be a promising therapeutic method for osteoporosis in the future.

Keywords: MSCs, exosomes, osteoporosis, therapeutic.

1. INTRODUCTION

Osteoporosis is a common disease characterized by a systemic impairment of bone mass and microarchitecture, results in increasing the propensity of fragility fractures [1]. However, the etiology of osteoporosis is complex and not completely known so far.

The pathogenesis of osteoporosis-related to the improper balance between the activities of osteoblasts and osteoclasts [2]. In addition, since osteoblasts and adipocytes share a common precursor, during the process of aging, the cell lineage commitment of MSCs shifts to adipocyte in bone marrow, resulting in osteoporosis [3]. Osteoporosis and angiogenesis are also intimately related. Accumulating evidence from studies in animals, cells and patients suggest that the local blood supply or decreased angiogenesis contributes to the process of osteoporosis [4]. Inflammation is a defensive mechanism for pathogen clearance and maintaining tissue homeostasis. In the skeletal system, inflammation is closely related to many bone disorders including osteoporosis [5]. MiRNAs are a class of short non-coding RNA molecules that regulate gene expression by targeting mRNAs [6]. They are involved in various cellular and molecular activities and played important roles in lots of biological and pathological processes [7]. Recently, miRNAs have been proved to play crucial roles in the etiology of various diseases such as osteoporosis [8].

MSCs have been proposed as promising candidates for a variety of therapeutic applications, such as bone, and cartilage regeneration, acute renal failure and neurological diseases, etc. [9]. Recently, it has been identified that MSCs exert their therapeutical activity through a paracrine effect, mediated by the release of small particles like growth factors [10]. Extracellular vesicles (EVs) released from MSCs are involved in tissue regeneration and contribute to the paracrine effect of MSCs [11].

EVs are membrane-packed vesicles and come in several different vesicular formats [12]. Mammalian cells can release different types of EVs. The main types include apoptotic bodies, microvesicles and exosomes [13]. The largest EVs (1000–5000 nm) are apoptotic bodies, which result from the fractionation of the cellular content of cells that die by apoptosis

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[14]. Microvesicles are shed directly from the cell surface membrane and have a diameter range of 500–1000 nm [15]. Exosomes comprise one of the main subclasses of EVs and have an endosomal origin [16]. Exosomes are small (30-100nm) extracellular nano-sized vesicles that originate as the internal vesicles of multivesicular bodies in many cell types [17], such as tumor cells, T-cells, mast cells and dendritic cells, etc. [18]. Recent findings uncovered that exosomes have a large variety of therapeutic applications in some disorders such as inflammatory diseases, cardiovascular diseases, metabolic and neurodegenerative disorders, etc. [19].

2. PROPERTIES OF OSTEOPOROSIS AND MSCs-DERIVED EXOSOMES

2.1. Properties of Osteoporosis

Osteoporosis is a systemic skeletal disease, characterized by low bone mineral density (BMD) and microarchitectural deterioration of bone tissues, with a consequent increase in susceptibility to fracture [20]. Owing to the changes of micro-architectural in trabecular and cortical skeleton, osteoporosis is manifested by fractures and enhanced skeletal fragility [21]. Hip and vertebral fractures are the most devastating consequences of osteoporosis and are closely related to the increased morbidity and mortality [22]. It is a worldwide health problem with serious consequences of personal suffering and economic costs [23]. The pathophysiology of osteoporosis is multifactorial. Such as the imbalance between osteoclastic bone resorption and osteoblastic bone formation [24]; decreased osteogenic differentiation and increased adipogenic differentiation [25]. At the same time, angiogenesis and inflammation also affect the pathogenesis of osteoporosis [26, 27]. Furthermore, deregulation of miRNAs mediated mechanisms is also becoming an important pathological factor in bone-related diseases such as osteoporosis [10].

2.2. Properties of Exosomes Derived from MSCs

In the recent years, many studies indicate that paracrine signaling by MSCs become a potential mechanism to explain the effects of these cells on tissue regeneration [28]. The paracrine effects were mediated by numerous factors such as extracellular vesicles, including exosomes. Exosomes are the smallest subset of EVs secreted by most cell types, such as MSCs, dendritic cells, macrophages, epithelial cells, B cells and T cells, etc. [29]. They can also be identified in the most bodily fluids including blood, urine, amniotic fluid, serum, etc. [30]. Similar to the cells, exosomes are composed of a lipid bilayer and can contain many molecular constituents of a cell, such as proteins, miRNAs, and DNA, etc. [31]. Tetraspanins (e.g., CD9, CD63, CD81) have been used as characteristic markers of exosomes [32]. Currently, the most common methods to isolate exosomes include ultracentrifugation and immune-bead isolation [33]. We can discover *via* the electron microscopy that exosomes are vesicles with deflated football or distinctive cup shaped morphology [34] (Fig. **1**) [35, 36].

2.3. Genetic Modification and the Secretion of Exosomes from MSCs

To secrete exosomes, several cellular steps need to be completed. Firstly, the formation of intraluminal vesicles (ILVs) in multivesicular bodies (MVBs); secondly, transport

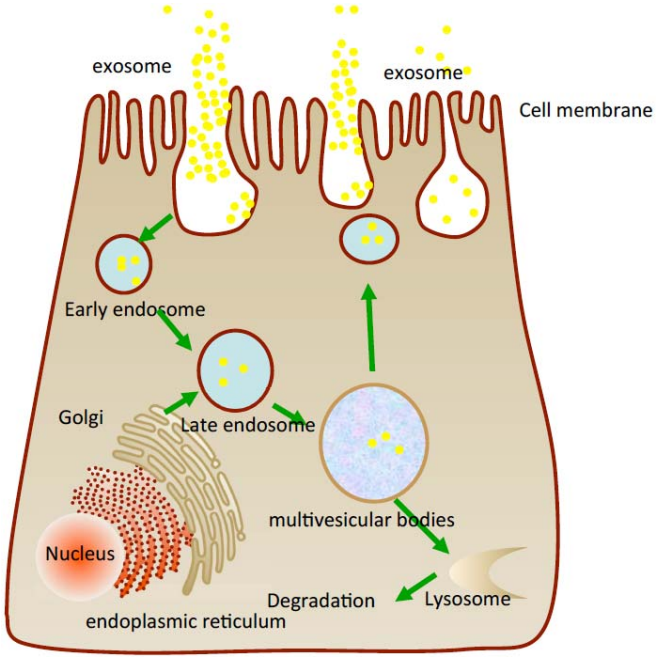

Fig. (1). Exosome biogenesis: exosomes are incorporated into multivesicular bodies. They could be targeted for lysosomal degradation or can be fused to cell membrane.

the MVBs to plasma membrane; third, fusion MVBs with the plasma membrane [37]. In this process, the endosomal sorting complex required for transport (ESCRT) machinery is very important [38]. ESCRTs consist of approximately twenty proteins that assemble into four complexes (ESCRT-0, -I, -II and -III) with associated proteins (VPS4, VTA1, ALIX, CHMP4C) [39]. *Via* the thorough RNAi screen study, seven ESCRT proteins can affect the secretion of exosomes [37]. Depletion ESCRT-0 proteins Hrs, TSG101 and ESCRT-I protein STAM1 could reduce exosomes secretion [37, 39]. Genetic modification might enhance the secretion of exosomes. For example, knockdown of the ESCRT-III and associated proteins VPS4B, VTA1, ALIX and CHMP4C could increase exosome secretion [37]. The ESCRT-0, ESCRT-I, ESCRT-III and related proteins VPS4B etc. are important for the secretion of exosomes. So we could modify these genes in order to regulate the secretion of exosomes from MSCs.

MSCs secrete and synthesize exosomes that are cholesterol rich phospholipid vesicles [40]. Compare to transplantation of exogenous MSCs, MSCs-derived exosomes are less immunogenic, easier to store and deliver than MSCs [41]. Searching for MSCs-derived exosomes is an attractive scope of the investigation because they are involved in cell-to-cell interactions [42]. Recent studies demonstrate that MSCs-derived exosomes can modulate the immune response and regenerate tissues such as heart and nerve *in vivo* [43].

3. ROLES OF MSCS-DERIVED EXOSOMES IN THE THERAPY OF OSTEOPOROSIS

3.1. MSCs-derived Exosomes and Osteogenesis

Osteogenesis is a key process responsible for the pathogenesis of osteoporosis, promoting osteogenesis is important for the treatments for osteoporosis [44]. Qi showed that

MSCs-derived exosomes could enhance ALP activity and up-regulated mRNA and protein expression of osteoblast genes in rBMSCs-OVX *in vitro*. At the same time, MSCs-derived exosomes can also stimulate bone regeneration in critical-sized calvarial defects in ovariectomized rats [45]. Furthermore, MSCs-derived exosomes can bind to matrix proteins such as type I collagen, fibronectin and induce osteoblastic differentiation *in vitro* and *in vivo* [43]. Bioactive materials such as Tricalcium Phosphate (β-TCP) provide an alternative solution for the repair of bone defects recently [46]. However, although β-TCP is osteoconductive and biocompatible, further improvements to osteogenesis are needed [47]. Interestingly, when combined with the β-TCP scaffolds besides MSCs-derived exosomes, osteogenesis activity of β-TCP can be enhanced through activating the PI3K/Akt signaling pathway [28, 48]. Given the osteogenic capacity of the MSCs-derived exosomes *in vitro* and *in vivo*, the exosomes derived from MSCs might be promising vesicles to improve bone formation in skeletal disorders such as osteoporosis [49].

3.2. MSCs-derived Exosomes and Osteoclast Differentiation

Normally, osteoporosis results from an imbalance of bone resorption and bone formation, where in the net activities of the osteoclasts supersede the osteoblasts [50]. Thus, understanding the mechanisms of MSCs-derived exosomes regulate osteoclast formation may be useful for the treatment of osteoporosis. MSCs release exosomes which contain functional miRNAs, and then be transferred from cell-to-cell by exosomes uptake and release, resulting in cross-cellular gene-regulation [51]. For example, human bone MSCs-derived exosomal miR-148a has been shown to suppress V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MAFB) expression and promote osteoclastogenesis [52]. The finding suggests that MSCs-derived exosomes might be an important component to regulate osteoclast differentiation in the treatment of osteoporosis. However, due to researches focus on MSCs-derived exosomes influence osteoclast differentiation are few recently, so comprehensive studies are necessary in the future.

3.3. MSCs-derived Exosomes and Angiogenesis

Angiogenesis is the process which new vasculature sprouts from pre-existing blood vessels [53]. Mounting evidence suggests that local blood supply or decreased angiogenesis play important roles in osteoporosis [54]. Therefore, basic strategy for enhancing osteoporotic bone regeneration is to promote angiogenesis [55]. Various studies have demonstrated the effect of MSCs-derived exosomes on key steps in angiogenesis [56]. Zhang J *et al*. found that hiPSC-MSCs-derived exosomes could promote angiogenesis and collagen synthesis in fibroblasts in HUVECs directly [57]. Similarly, another study concluded that iMSCs-derived exosomes could activate angiogenesis-related molecule expression (such as PGF, HIF-1α, TGFB1, *etc.*) and promote HUVECs migration and tube formation [58]. Moreover, angiogenesis plays crucial roles in numerous physiological processes, it requires a tight interaction between endothelial cells and their surrounding environment. MSCs-derived exosomes might repressed the expression of the angiogenic inhibitor delta-like 4 (DLL4) by targeting its 3′ untranslated region [58]. MSCs-derived exosomes could modulate endothelial cell angiogenesis by promoting the formation of endothelial tip cells. In conclusion, MSCs-derived exosomes as a pro-angiogenic factor might be a promising candidate for the treatment of osteoporosis [59].

3.4. MSCs-derived Exosomes and Adipogenesis

In bone marrow, osteoblasts and adipocytes share a common precursor MSCs [60, 61]. Accumulating information show that osteoporosis shifts the MSCs fate to favor adipocytes over osteoblasts [62], and decrease bone mass with marrow fat accumulation [63]. Martin *et al*. found that human mesenchymal stem cells (HMSCs) derived adipocytes could secrete exosomes containing adipogenic specific mRNAs (such as leptin, PPARg, CEBPa and CEBPd transcripts) and antiosteogenic miRNAs (miR30c, miR-31, miR125a, miR-125b, miR-138) that can be transferred to MSCs-derived osteoblasts [64]. MiR-30c and miR-31, targeting osteogenic transcripts RUNX2 and Osterix [65, 66], regulate the osteogenic differentiation. MiR-125a and miR-125b are significantly down-regulated during osteogenic differentiation in human adipose-derived stem cells [67]. MiR-138 modulates osteogenic differentiation of hMSCs, overexpression of miR-138 reduced bone formation [68]. MiR-30c, miR-31, miR-125a, miR-125b and miR-138 were selected for their capacity to inhibit osteoblast gene expression, all of them showed an increase in their expression in the research [64]. These results probably implicated that the miRNAs in exosomes might repress osteogenic differentiation. These findings indicate that MSCs-derived exosomes might be a target component to regulate the relationship between osteoblasts and adipocytes in the treatment of osteoporosis.

3.5. MSCs-Derived Exosomes and Inflammation

The process of bone repair requires inflammatory response, as the immune system responds to a variety of cytokines that recruit and activate several cell types, like MSCs, to promote bone formation [69]. Exosomes secreted from MSCs have been reported to contribute to the regulation of the immune system [70]. Dysregulated inflammation leads to the increased bone resorption and suppressed bone formation [71]. T cells have been identified as key regulators of osteoclast and osteoblast formation and activity in different diseases, such as osteoporosis [72]. MSCs-derived exosomes have an inhibitory role in the differentiation, proliferation, and activation of T cells [73]; MSCs-derived exosomes might regulate the immune system and influence bone metabolism, suggesting potential clinical application of MSCs-derived exosomes in cell-free therapy of osteoporosis.

3.6. MSCs-derived Exosomes and miRNAs

There are growing evidence suggesting that a number of exosomal-containing miRNAs obtained from MSCs can regulate bone metabolism [74]. Using isolated MSCs-derived exosomes, a number of miRNAs (for example, miR-135b, miR-148a, miR-199b, miR-218 and miR-221) were identified to be up-regulated during MSCs culture, while some miRNAs (such as miR-155, miR-181a, miR-221, miR-320c

Table 1. MSCs-derived exosomes miRNAs and their potential effects on bone metabolism.

MiRNA	Regulate Gene	Effects	References
let-7	HMGA2	Enhance ostegenesis/repress adipogenesis	[76]
miR-199b	Runx2	Control osteogenic	[77]
miR-218	Wnt	Enhance osteogenesis	[78]
miR-135b	IBSP/ Osterix	Repressing osteogenesis	[79]
miR-221	Runx2	Rrepressing osteogenesis	[52]
miR-148a	MAFB	Promote osteoclastogenesis	[52]

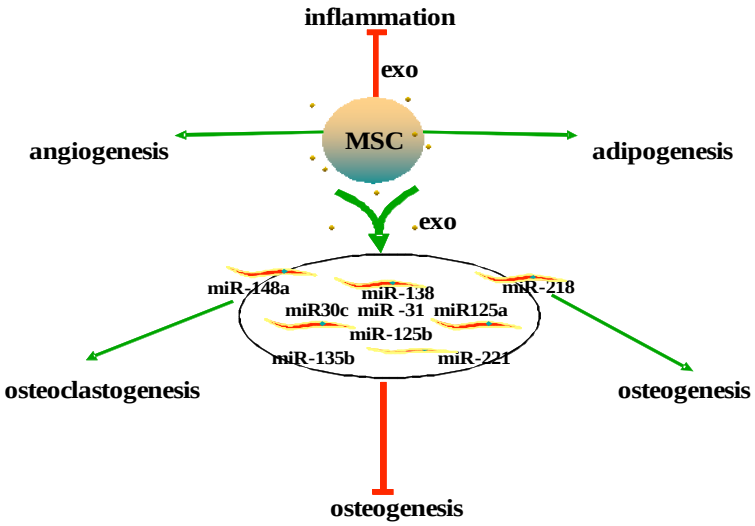

Fig. (2). MSCs-derived exosomes and the relationship with osteogenesis, osteoclast differentiation, angiogenesis, immune processes and miRNAs.

and miR-885-5p) were shown to be down-regulated [52, 75]. MiRNA let-7 was reported to enhance osteogenesis while repressing adipogenesis of HMSCs by regulating HMGA2 [76]. MiR-199b was shown to regulate Runx2 to control the osteogenesis [77]. MiR-218 stimulates the Wnt pathway to promote MSCs osteogenesis [78]. MiR-135b reduced the expression of osterix and integrin binding sialoprotein (IBSP), suppressed MSCs osteogenic differentiation [79]. Down-regulation of miR-221 was discovered influence Runx2 expression and trigger osteogenesis in HMSCs [52]. Therefore, future investigation will be focussed on the potential function of miRNAs derived from MSCs exosomes in bone metabolism (Table **1**) (Fig. **2**) [80].

4. THE ADVANTAGE AND CHALLENGE OF MSCs-DERIVED EXOSOMES FOR OSTEOPOROSIS THERAPY

In the past few years, MSCs-derived exosomes have shown exciting promise and great ability to provide therapeutically benefits for diseases such as rheumatoid arthritis, osteoarthritis, etc. [81]. Use of MSCs-derived exosomes has several potential advantages. First, their usage avoids the transfer of cells that might have mutated or damaged DNA [82]; Second, the vesicles are small and circulate readily whereas MSCs are too large to circulate [82]; Thirdly, exosomes do not contain MHCI proteins and can overcome some disadvantages of cell transplantation therapies. Researchers have shown that application to xenogeneic animals did not induce obvious immune reactions [45].

Moreover, compared to traditional MSCs therapies, exosomes therapies might decrease injury from MSCs transplantation surgery and the possibility of favoring tumor growth by MSCs [40]. Furthermore, exosomes have advantages over the corresponding MSCs. MSCs-derived exosomes are less complex than cells, so they are easier to produce and also have the potential to avoid some of the regulatory issues that face in MSCs [83]. Therefore, MSCs-derived exosomes might represent an ideal therapeutic tool for bone diseases like osteoporosis in the near future.

However, despite the interesting possibilities for MSCs-derived exosomes treatment, novel therapeutic approaches still face some challenges. First, although remarkable progress have been made in the development of exosomes isolation techniques, it has also been proven that it is challenging to rapidly and efficiently isolate exosomes. Because of the complexity of biological samples and the heterogeneity of exosomes themselves [84]. Second, due to the complex structure of MSCs-derived exosomes, it might be difficult to characterize pharmaceutically [85]. Third, the question of which cell type to be used for exosomes derivation still re-

mains to be answered [86]. Hence, the novel therapeutic approaches still need comprehensive investigation in order to bring them into osteoporosis applications.

CONCLUSION

Osteoporosis has become a serious threat to elderly people both at the individual and society levels. The recent findings suggest that MSCs-derived exosomes might one day be able to provide an effective therapy for bone metabolism disease like osteoporosis. The molecular mechanisms of the effect by MSCs-derived exosomes on osteogenesis, osteoclast differentiation, angiogenesis, angiogenesis and immune processes and miRNAs have been reported here, however, the exact mechanism still unknown and has to be studied further. Meanwhile, considering the majority of the MSCs-derived exosomes studies are currently on pre-clinical stage and the conventional methods for isolating and characterizing exosomes are not effective for clinical application. Therefore, it is important to develop a large scale of exosomes production, as well as isolation and purification methods. Moreover, further investigations are required to explore specific mechanisms of generation, secretion and action of MSCs-derived exosomes. Finally, we believe that exosomes could be a promising new therapeutic strategy for osteoporosis although several challenges we might face.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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