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Original Article

Apoptotic vesicles inhibit bone marrow adiposity via wnt/β-catenin signaling

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ABSTRACT

Background: There is currently increasing focus on aging-related diseases. Osteoporosis is a common disease the incidence of which increases with age. In older patients with osteoporosis, bone marrow mesenchymal stem cells (BMMSCs) have a decreased capacity for osteogenesis and an increased capacity for adipogenesis, causing excessive accumulation of adipose tissue in the bone marrow. Therefore, means of reducing bone marrow adiposity may have therapeutic potential for osteoporosis. Apoptotic vesicles (apoVs) participate in a wide range of physiological processes and have been shown to have therapeutic effects in a variety of diseases. The principal objective of this study was to examine the special properties and regulatory mechanisms of BMMSC-derived apoVs in the treatment of bone marrow adiposity.
Results: The results showed that apoVs could decrease bone marrow adiposity in osteoporotic mice and prevent adipogenic differentiation of MSCs by activating the Wnt/β-catenin pathway.
Conclusion: New apoV-based therapies have potential for the treatment of bone marrow adiposity in patients with aging-related osteoporosis and may be further applicable to the treatment of obesity and aging-related diseases.

1. Introduction

Aging is accompanied by an increase of adipocytes in the bone marrow cavity, which can affect bone formation [1,2]. Osteoporosis, characterized by low bone mass and excessive buildup of adipose tissue in the bone marrow microenvironment, is a common aging-related bone disease [3,4]. The dynamic balance of osteogenic/adipogenic differentiation of bone marrow mesenchymal stem cells

Abbreviations: AM, Adipogenic medium; ApoVs, Apoptotic vesicles; BMMSCs, Bone marrow mesenchymal stem cells; BMP, bone morphogenic proteins; C/EBPα, CCAAT/enhancer-binding protein α; EVs, Extracellular vesicles; FBS, Fetal bovine serum; GAPDH, Glyceraldehyde3-phosphate dehydrogenase; H&E, Hematoxylin and eosin; MSCs, Mesenchymal stem cells; NTA, Nanoparticle tracking analysis; OVX, ovariectomy; PM, Proliferation medium; PPARγ, Peroxisome proliferator activated receptor γ; RT-qPCR, Real-time quantitative polymerase chain reaction; STS, Staurosporine; T2DM, type 2 diabetes mellitus; TEM, Transmission electron microscopy; TGF-β, transcriptional growth factor-beta; Wnt, wingless-type MMTV integration site.

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(BMMSCs), common precursors of osteoblasts and adipocytes, is closely related to the development of skeletal diseases [5,6]. In response to other pathological stimuli, such as aging or hormonal disorders, BMMSCs preferentially differentiate toward adipocytes, leading to progressive bone loss and increased bone marrow adiposity [7]. These changes in bone microarchitecture lead to increased bone fragility and fracture susceptibility. Therefore, reducing bone marrow adiposity may be essential for the treatment of osteoporosis.

Extracellular vesicles (EVs) can be produced by almost all cells through a wide range of mechanisms. EVs mainly consist of exosomes, microvesicles, and apoptotic vesicles (apoVs) [8,9]. EVs, which are essential for cell and tissue communication, are abundant in protein, RNA, and lipids [10–12], and have a range of biological functions, including roles in the responses to infections, tumors, and cardiovascular diseases [13–15]. EVs have also been shown to regulate the differentiation of stem cells [16,17]. Recent studies have focused on the regulation of physiological processes by apoVs, apoptotic bodies could maintain MSC homeostasis and ameliorate osteopenia [18], apoptotic EVs could promote osteochondral regeneration by enhancing stem cell activity and regulating immunity [19]. We also demonstrated that MSC-derived apoVs could ameliorate haemophilia A via activating platelet functions [20], and our previous studies showed that BMMSC-derived apoVs can regulate bone metabolism, but their role in regulating bone marrow adiposity has not been investigated [21,22]. This study examined the role of BMMSC-derived apoVs in mesenchymal stem cell (MSC) adipogenesis and the underlying regulatory mechanisms *in vivo* and *in vitro*.

In aging-related osteoporosis, a reduction in bone mass is frequently accompanied by an increase in adiposity in the bone marrow cavity [23–25]. The activation of key signaling pathways and transcription factors regulates BMMSC differentiation toward osteogenesis and adipogenesis [26]. Signaling pathways, including wingless-type MMTV integration site (Wnt) [27], bone morphogenic proteins (BMPs)/transcriptional growth factor-beta (TGF-β) [28], Notch [29], Hedgehog [30], and others [31], regulate stem cell differentiation toward osteogenesis and adipogenesis. Activation of Wnt/β-catenin signaling inhibits stem cells from differentiating into adipocytes during bone formation and increases bone mass by promoting osteoblast development, stem cell renewal, suppression of osteoblast differentiation, and osteoclast apoptosis [27,32,33]. Wnt/β-catenin signaling mainly inhibits stem cell adipogenic differentiation by inhibiting CCAAT/enhancer-binding protein α (C/EBPα) and peroxisome proliferator activated receptor γ (PPARγ) and increasing the expression of Runt-related transcription factor 2, Distal-less Homeobox 5, and Osterix, leading to stem cell differentiation toward osteoblasts, thus regulating bone marrow adiposity [34,35]. Therefore, this study was performed to examine the role of the Wnt/β-catenin pathway in regulating bone marrow adiposity in aging-related diseases.

2. Methods

All the reagents and sources were listed in Table S1.

2.1. Culture and adipogenic induction of MSCs

The MSCs were obtained from ScienCell and cultured in α-minimum essential medium (α-MEM). The proliferation medium (PM) was prepared by supplementing α-MEM with 10 % (*v*/*v*) fetal bovine serum (FBS) and 1 % (*v*/*v*) antibiotics to support cell growth. The adipogenic medium (AM) consisting of 10 % (*v*/*v*) FBS, 1 % (*v*/*v*) antibiotics, 50 nM insulin, 100 nM dexamethasone, 500 μM 3-isobutyl-1-methylxanthine, and 200 μM indomethacin in α-MEM was used to induce the adipogenic differentiation of MSCs.

2.2. TUNEL staining

The PM used to culture the MSCs was replaced with serum-free medium. Apoptosis was induced by treating the cells with staurosporine (MedchemExpress, Monmouth Junction, NJ, USA) for 24 h. To confirm apoptosis, normal or apoptotic BMMSCs were fixed with 0.2 % Triton X-100 (Solarbio) on confocal culture dishes. Apoptosis was detected using a TUNEL Cell Apoptosis Detection kit (Applygen, Beijing, China). Fluorescence images were captured using confocal laser scanning microscopy (CLSM; Carl Zeiss, Germany).

2.3. Isolation, purification, and identification of apoVs

ApoVs were collected as previously described, with some modification [20, 21]. The concentration of proteins in apoVs was determined using a bicinchoninic acid (BCA) kit (Thermo Fisher Scientific). The apoV sample was carefully dropped onto a carbon-coated copper grid. The sample was allowed to air dry and then imaged using a transmission electron microscope (TEM). To determine the particle size, the apoVs suspension at the appropriate concentration was added to the inlet tube and measured by nanoparticle tracking analysis (NTA). Membrane surface proteins of ApoVs were determined by immunoelectron microscopy. ApoVs were immobilized on a copper grid and the distribution of colloidal gold nanoparticles was observed in a TEM after incubation with primary antibody and secondary antibody attached to the colloidal gold nanoparticles.

2.4. Western blotting analysis

Briefly, apoV or MSCs were lysed to extract total protein, and its concentration was quantified using the BCA method. Protein extracts were analyzed by 10 % SDS-PAGE and proteins were transferred onto PVDF membranes, blocked at 25 °C for 1 h. Then membranes were incubated with primary antibodies overnight followed by incubation with corresponding horseradish peroxidase-conjugated secondary antibodies at room temperature. An enhanced chemiluminescence kit was used to detect the protein bands.

2.5. Animals and administration

To generate a mouse model of estrogen deficiency-induced bone loss, 8-week-old female mice underwent bilateral oophorectomy. Following surgery, the mice were randomly assigned to one of two groups: the ovariectomy (OVX) group or the ovariectomy plus apoptotic vesicles (OVX + apoVs) group (n = 10 mice per group). Other 8-week-old mice underwent a sham operation without removal of the ovaries as controls.

In a separate experiment, 18-month-old female mice were randomly divided into either the aged control group or the aged + apoVs group (n = 10 mice per group). Mice in the aged + apoVs group received intravenous injections of apoVs via the tail vein.

2.6. ApoV uptake by MSCs

The uptake of apoV by MSCs was monitored using the PKH-26 kit (Umibio, Co. Ltd., Shanghai, China) at different time points. A mixture containing PKH-26 and apoVs was centrifuged at 16000×*g* for 30 min. PKH-26-labeled apoVs was incubated with MSCs for 10 min. The cells were fixed, permeabilized with 0.1 % Triton X-100,

washed twice with PBS, and then stained with 5 μg/mL fluorescein isothiocyanate (FITC). Nuclei were stained with 4′,6-diamidine-2-phenylindole (DAPI).

2.7. Oil red O staining and quantification

MSCs were cultured in different media for 14 days, stained with Oil red O, and their morphology was observed using an optical microscope (Olympus, Tokyo, Japan). The stained cells were added to 100 % isopropyl alcohol, and the absorbance at 500 nm was measured using a microplate reader.

2.8. Real-time quantitative PCR

Total RNA was extracted using TRIzol reagent (15596026, Invitrogen) and the quality and concentration of the extracted RNA were assessed using a spectrophotometer. cDNA was synthesized with a PrimeScript RT Reagent Kit. Quantitative PCR was performed in a 10 μL reaction volume containing cDNA template, gene-specific primers, and SYBR Green or TaqMan master mix. The reverse transcription real-time quantitative polymerase chain reaction (RT-qPCR) was performed on an ABI Prism 7500 Real-Time PCR System. All reactions were performed in triplicate to ensure accuracy and reproducibility of results. The primer sequences are listed in Table S2.

2.9. Ectopic adipose tissue formation

Aliquots of 1×10^6 MSCs were mixed with collagen membrane scaffold, and ectopic adipose tissues were harvested after 6 weeks. For histological evaluation, sections were stained with hematoxylin and eosin (H&E) and Oil red O to assess tissue structure and general morphology. The stained sections were examined using an optical microscope to evaluate the presence and distribution of ectopic adipose tissues within the collagen scaffold.

2.10. Statistical analysis

Statistical analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA). The Shapiro–Wilk test was employed to assess the normality of data distribution, with a P value > 0.05 indicating a normal distribution. Comparisons between two groups were performed using independent two-tailed Student's t tests, while comparisons involving more than two groups were analyzed using one-way analysis of variance (ANOVA) and Tukey's post hoc test. All data are expressed as the mean ± standard deviation (SD), and a P value < 0.05 was considered statistically significant.

3. Results

3.1. Extraction and characterization of BMMSC-derived apoVs

Apoptosis is an active programmed cell death process that generates large numbers of apoVs [36–38]. Here, we used staurosporine (STS) to induce apoptosis of BMMSCs. The STS group had more TUNEL-positive stained cells (red) compared with the sparsely stained cells in the control group (Fig. 1A). ApoVs were extracted according to the procedure shown in Fig. 1B—as described in detail in our previous reports [21,39]. The diameter of apoVs was approximately 200 nm, as determined by TEM (Fig. 1C). NTA showed that the diameter distribution range of apoVs was 172.7 ± 0.2 nm (Fig. 1D). Western blotting showed CD9, CD63, CD81, Fas, and integrin alpha-5 were present in apoVs, which was is consistent with previous studies [20,40] (Fig. 1E), the original figures of western blotting were shown in Fig. S3. Immunoelectron microscopy showed the expressions of CD9 of apoVs, which was also a method commonly used to detect apoptotic vesicles (Fig. 1F).

3.2. BMMSC-derived apoVs decreased bone marrow adiposity in aged mice

EVs are commonly injected via the tail vein in rodent models to examine their potential efficacy in disease [17,41]. These EVs enter the inferior vena cava via the caudal vein and then flow sequentially through the heart, aorta, and all levels of branching arteries for rapid distribution throughout the body [42]. Fluorescently labeled exosomes were injected via the tail vein of mice and shown by live imaging to be captured mainly by the liver, spleen, lung, kidney, and bone marrow, with the liver, spleen, and lung showing the strongest fluorescence [43]. To detect the distribution of apoVs in the major organs of mice, we injected apoVs labeled with the near-infrared fluorescent cyanine dye 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide (DiR) into mice via the tail vein. At 24 h and 48 h after injection, the fluorescent signal was detected using a whole-body fluorescence imaging system (Fig. S1). The signal was mainly concentrated in the liver, indicating that a large proportion of apoVs were metabolized by the liver following injection via the tail vein. The images also showed the presence of apoVs in the bone.

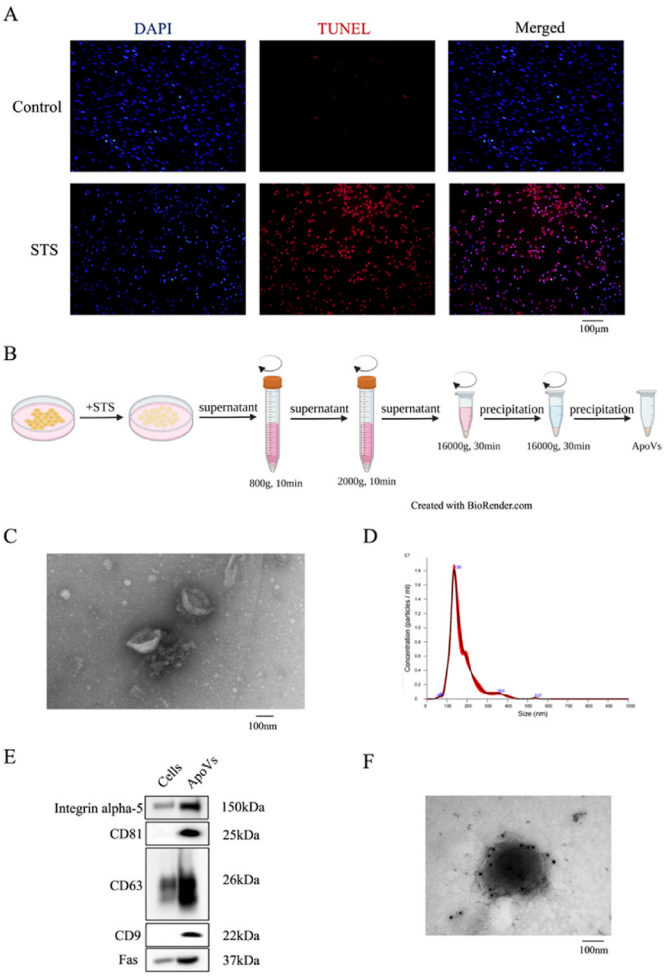

Fig. 1. Characterization of BMMSC-derived apoVs. **A**. TUNEL staining. **B**. Schematic of apoV extraction. **C**. Morphology of apoVs observed by TEM. **D**. Particle size distribution of apoVs. **E**. Western blotting analysis. **F**. Immunoelectron microscopy showed the expressions of CD9 of apoVs.

Our previous study showed that BMMSC-derived apoVs can regulate bone metabolism by promoting the osteogenic differentiation of MSCs and inhibiting osteoclast formation [21]. In addition, we found that BMMSC-derived apoVs inhibited bone marrow adiposity. First, we constructed a mouse model of estrogen deficiency and age-related osteoporosis. Then, the animals were administered apoVs via the tail vein eight times, once a week (Fig. 2A). H&E and Oil red O staining were performed to evaluate adipose tissue in the femur. H&E staining (Fig. 2B) and Oil red O staining (Fig. 2C) showed that apoVs significantly decreased adipose tissue formation in the femurs of aged mice. Adipocyte count (Fig. 2D) and adipocyte area/tissue area (Fig. 2E) determined by H&E staining of femurs showed that apoVs decreased adipose tissue formation. Postmenopausal osteoporosis is a common condition associated with aging in women [3]. H&E staining (Fig. S2A) and Oil red O staining (Fig. S2B) showed that apoVs significantly decreased adipose tissue formation in OVX mice. Adipocyte count (Fig. S2C) and adipocyte area/tissue area (Fig. S2D) showed that apoVs decreased adipose tissue formation in OVX mice.

3.3. BMMSC-derived apoV uptake by MSCs

Within the bone marrow of patients with osteoporosis, MSCs show more differentiation toward adipocytes and less differentiation toward osteoblasts [44,45]. Therefore, disorders in the regulation of MSC fate commitment may also play important roles in the mechanism underlying osteoporosis. To investigate whether MSCs could take up apoVs, MSCs were incubated with PKH26-labeled apoVs for 2–72 h. The number of red-stained particles around the nucleus increased gradually from 2 to 24 h, decreasing gradually thereafter, which may have been due to host metabolism (Fig. 3). EVs form endogenous transport systems involved in the exchange of biomolecules (proteins and RNAs) between cells, suggesting their potential for applications in drug delivery and regenerative medicine [46,47]. In future studies, we will also search for means of promoting uptake of apoVs by cells to facilitate the entry of apoV components into target cells to engineer more effective apoV-based therapeutic agents.

3.4. BMMSC-derived apoVs inhibited MSC adipogenesis in vitro and in vivo

We treated MSCs cultured in PM and AM with BMMSC-derived apoVs to further explore the effects on MSC adipogenesis. Oil red O staining (Fig. 4A) and quantification (Fig. 4B) showed that 0.4 μg/mL apoVs had the most pronounced inhibitory effect on MSC adipogenesis, as well as on the mRNA levels of *PPARγ* and *C/EBPα* (Fig. 4C). Therefore, we used 0.4 μg/mL apoVs in subsequent experiments. MSCs in AM were treated with or without apoVs and examined by Oil red O staining after 7 days of adipogenic induction. Oil red O staining (Fig. 5A) and quantification (Fig. 5B) showed that apoVs significantly inhibited MSC adipogenesis. *PPARγ* and *C/EBPα* expression was decreased when MSCs were treated with apoVs after 7 days of induction of adipogenesis (Fig. 5C). In addition, western blotting showed that PPARγ expression was downregulated during adipogenesis in the group treated with apoVs (Fig. 5D and E), the original figures of western blotting were shown in Fig. S4. H&E staining (Fig. 5F) and Oil red O staining (Fig. 5G) showed that there were fewer adipose tissue-like structures in the group treated with AM + apoVs compared to the group treated with AM. Therefore, BMMSC-derived apoVs inhibited MSC adipogenesis *in vitro* and *in vivo*.

3.5. BMMSC-derived apoVs inhibited MSC adipogenesis by activating Wnt/β-catenin signaling

The mechanisms underlying aging-related osteoporosis are complex and known to involve multiple factors [48,49]. BMMSCs

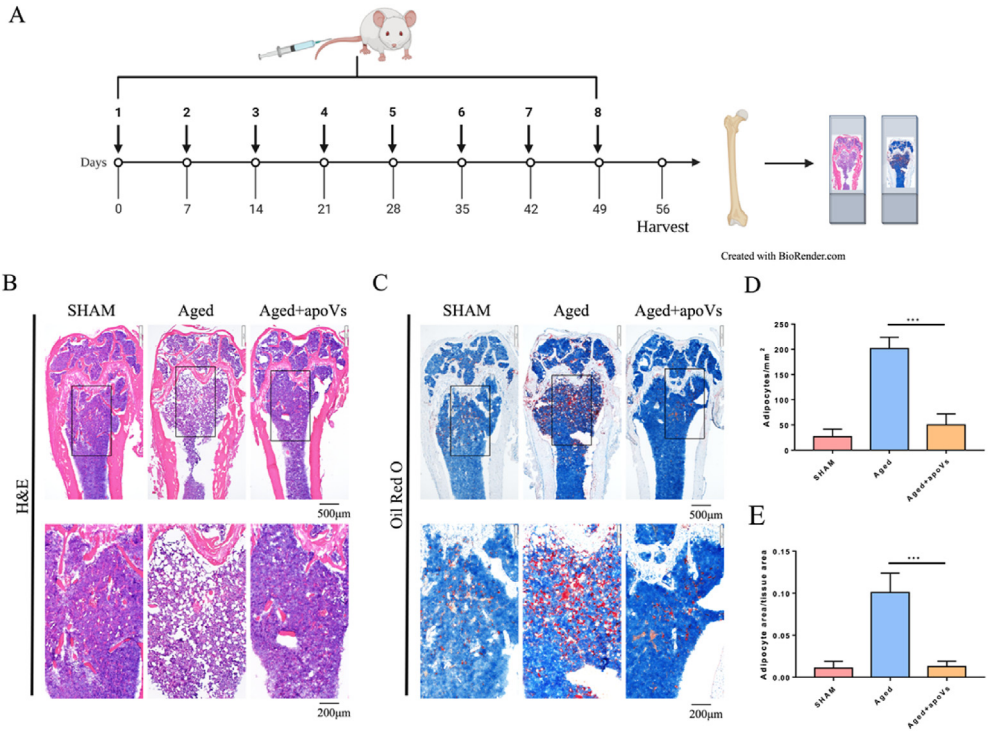

Fig. 2. BMMSC-derived apoVs decreased bone marrow adiposity in aged mice. **A.** Schematic of tail vein injection. **B.** H&E staining. **C.** Oil red O staining. **D.** Adipocyte count. **E.** Adipocyte area/tissue area. ***$P < 0.001$.

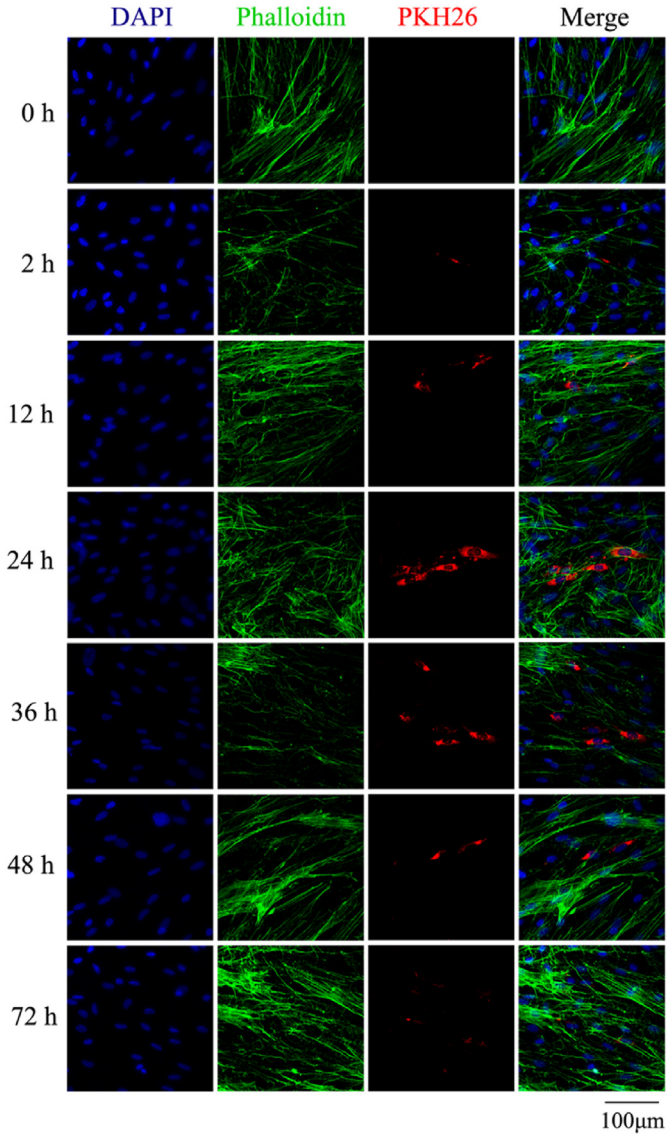

Fig. 3. Uptake of PKH26-labeled apoVs (red) by MSCs. The nuclei of MSCs were stained with DAPI (blue). The F-actin of MSCs was stained with phalloidin (green). The apoVs were labeled with PKH26 (red).

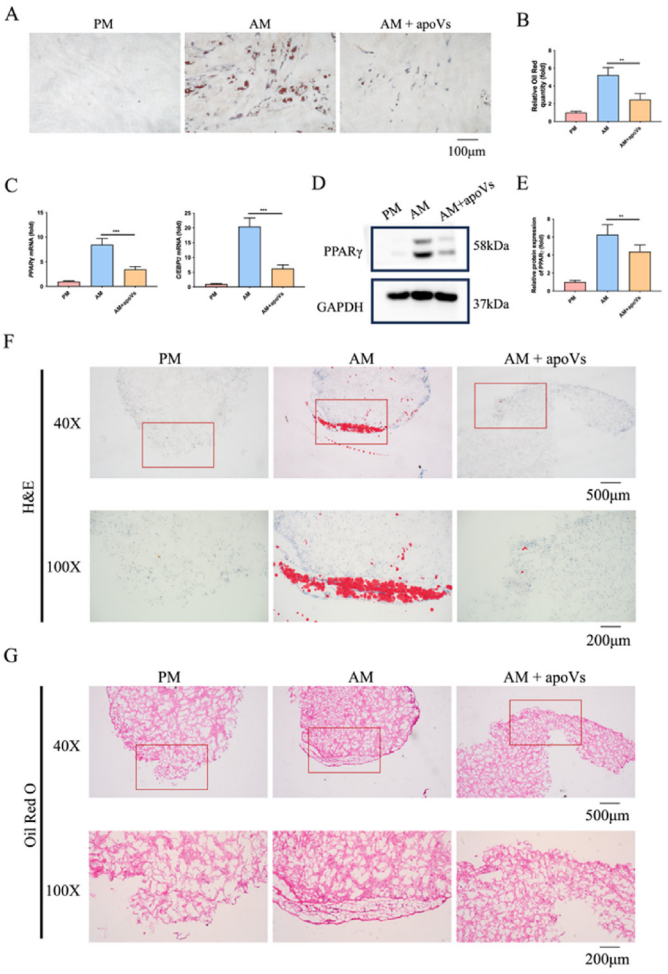

Fig. 5. BMMSC-derived apoVs inhibited MSC adipogenesis *in vitro* and *in vivo*. **A**. Oil red O staining. **B**. Oil red O quantification. **C**. *PPARγ* and *C/EBPα* mRNA expression on day 7. **D.** Western blotting of PPARγ. **E.** Semi-quantitative analysis of PPARγ protein expression. **F**. H&E staining (*in vivo*). **G.** Oil red O staining (*in vivo*). $^{**}P < 0.01$, $^{***}P < 0.001$.

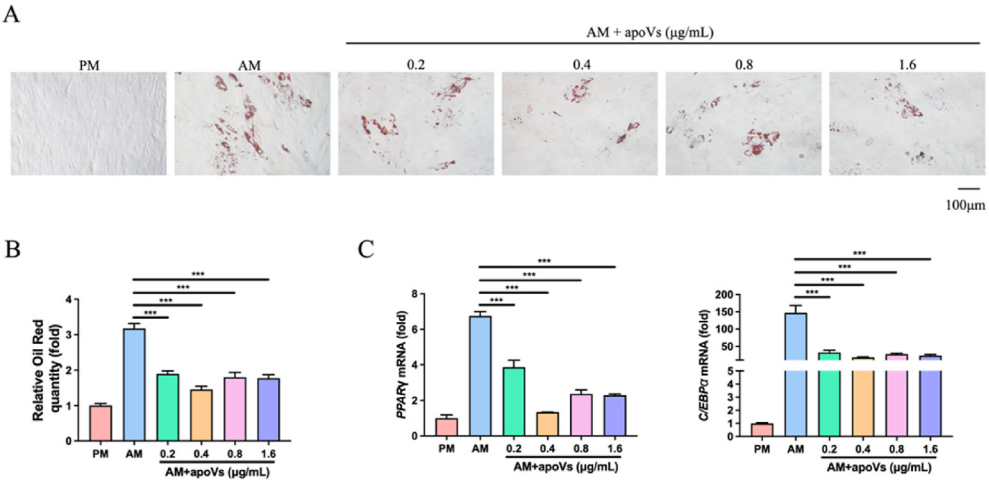

Fig. 4. Screening of apoVs for concentration yielding the highest activity. **A**. Effects of different concentrations of BMMSC-derived apoVs on MSC adipogenesis. **B**. Quantitative analysis of Oil red O staining. **C**. *PPARγ* and *C/EBPα* mRNA expression on day 7.

are common progenitors of osteoblasts and adipocytes. The dynamic balance of the osteogenic/adipogenic differentiation of BMMSCs is closely related to the development of osteoporosis [50]. Wnt/β-catenin signaling is involved in regulation of adipogenesis [51,52]; ectopic Wnt expression in preadipocytes activates Wnt/β-catenin signaling and prevents adipogenesis [53]. Determination of the means of activating the Wnt/β-catenin signaling pathway may contribute to the development of therapeutic treatments for bone marrow adiposity.

To clarify the mechanism by which apoVs regulate adipogenic differentiation of stem cells, we examined key proteins of the Wnt/β-catenin signaling pathway and found that apoVs increased expression of Wnt3a and β-catenin (Fig. 6A), the original figures of western blotting were shown in Fig. S5. Semiquantitative analysis of protein expression showed the same results, with apoVs significantly increasing the expression of Wnt3a and β-catenin (Fig. 6B). To further clarify the regulation of Wnt/β-catenin pathway by apoVs, we used the pathway agonist LiCl and the inhibitor PNU-74654 [52,54]. Western blotting analysis showed that activation of β-catenin by apoVs was further enhanced by LiCl, while PNU-74654 had an inhibitory effect (Fig. 6C), the original figures of western blotting were shown in Fig. S6. The results of semi-quantitative protein analysis were consistent with these findings (Fig. 6D). Subsequently, LiCl and PNU-74654 were added to MSCs for induction of adipogenesis. Histochemical analysis showed that MSC adipogenesis was reduced by the addition of LiCl, while the addition of PNU-74654 enhanced adipogenesis of MSCs compared with the group treated with apoVs (Fig. 6E). Oil red O quantification was consistent with these results (Fig. 6F). In addition, *PPARγ* and *C/EBPα* expression was significantly lower in the apoV-treated group than the group without apoV treatment. Compared with the group treated with apoVs alone, *PPARγ* and *C/EBPα* expression was significantly reduced by LiCl and significantly increased by PNU-74654 (Fig. 6G). The above results further confirmed that apoVs regulated MSC adipogenesis through the Wnt/β-catenin pathway (Fig. 7).

4. Discussion

Aging is a complex and progressive physiological process accompanied by the accumulation of damaged molecules that trigger aging-related diseases, such as osteoporosis, cardiovascular

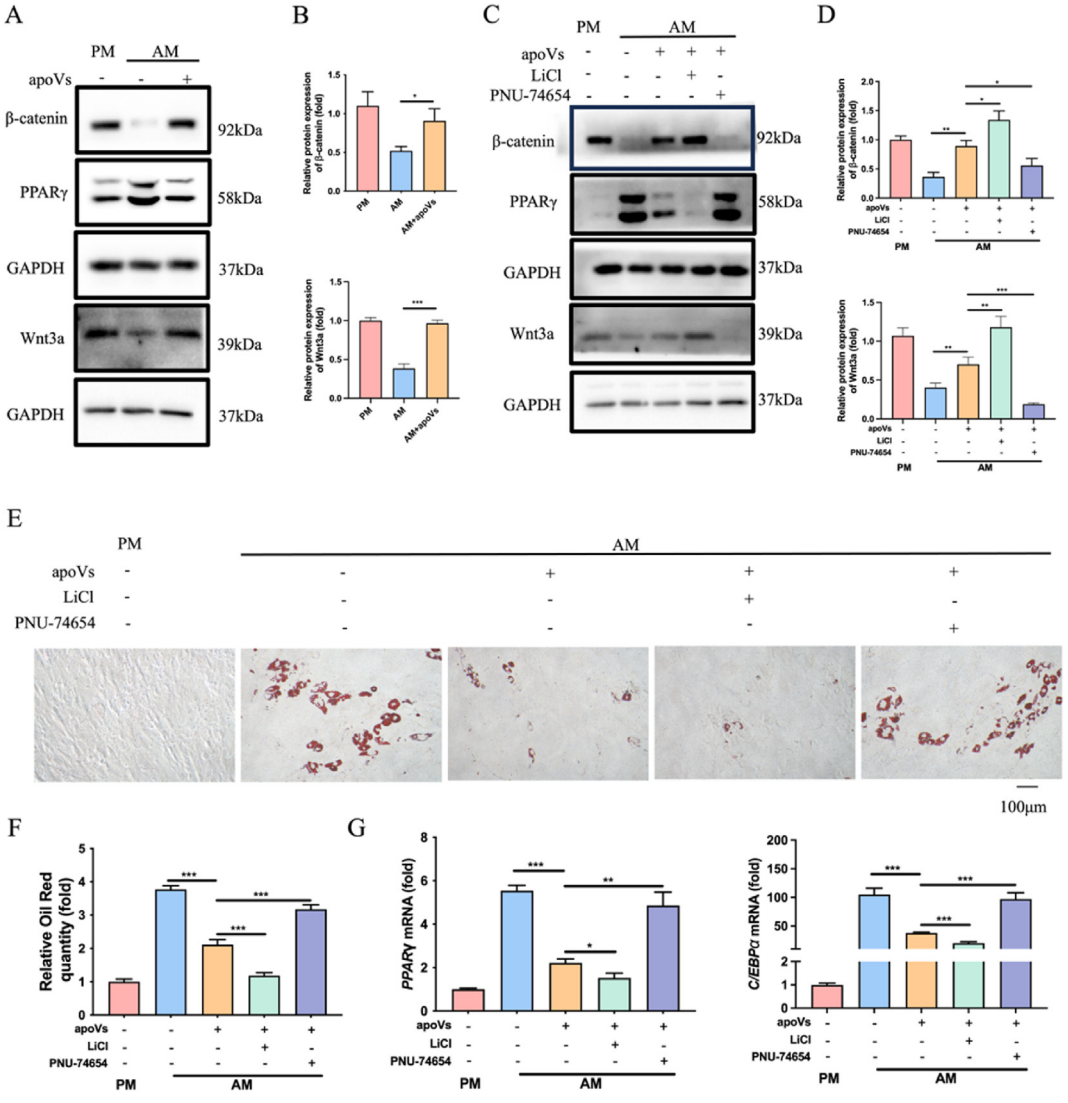

Fig. 6. ApoVs inhibited adipogenesis of MSCs by activating Wnt/β-catenin signaling. **A**. β-Catenin, PPARγ and Wnt3a expression were determined by western blotting. **B**. Quantitative protein analysis. **C**. β-Catenin, PPARγ and Wnt3a expression with the addition of agonists and inhibitors was determined by western blotting. **D**. Quantitative protein analysis. **E**. Oil red O staining. **F**. Quantitative analysis of Oil red O staining. **G**. *PPARγ* and *C/EBPα* mRNA expression on day 7. $^{*}P < 0.1$, $^{**}P < 0.01$, $^{***}P < 0.001$.

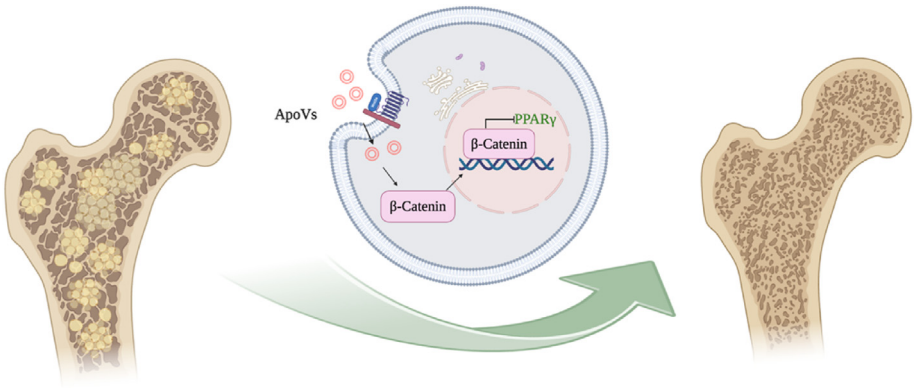

Fig. 7. Schematic representation of the inhibition of adipogenesis by apoVs in MSCs via activation of the Wnt/β-catenin signaling pathway.

disease, arthritis, T2DM, and Alzheimer's disease [55–57]. The self-renewal capacity of MSCs decreases with aging, leading to further organismal aging and pathophysiological aging-related phenomena [58]. Osteoporosis is associated with not only increased bone resorption but also dysfunction of MSCs, as evidenced by a shift from osteogenic to adipogenic differentiation of MSCs and a decrease in their self-renewal capacity [59,60]. Recent research has focused on the relationship between bone marrow adipose tissue and osteoporosis, and shown that abnormal expansion of marrow adipose tissue plays a critical role in the development and progression of osteoporosis [6,31,61].

Bone loss in osteoporosis is frequently accompanied by bone marrow adiposity [62]. Further studies showed that the dedifferentiation of BMMSCs caused by various factors results in excessive production of adipocytes and that the abnormal deposition of lipids alters the local microenvironment, which may in turn affect osteoblasts and inhibit the differentiation and maturation of osteoblasts, causing BMMSCs to further favor adipogenic differentiation [63–65]. Our results showed that apoVs reduced bone marrow adiposity in osteoporotic mice, suggesting their therapeutic potential for treatment of osteoporosis and possible further applications in the treatment of other aging-related diseases.

ApoVs are rich in proteins, RNA, and lipids [9], we conducted systematic investigations that focused on both the proteins and miRNA of apoVs in our earlier studies [20,22]. ApoVs could inherit functional molecules from parental cells and transport molecules, and our extensive research into the role of MSCs-apoVs would contribute to a better understanding of regeneration therapy. Recent studies have suggested apoVs have therapeutic potential for a variety of diseases [66–68]. Kou et al. reported that MSC-derived apoptotic extracellular vesicles could induce apoptosis and inhibit growth of multiple myeloma cells [66]. Kou et al. showed that MSC-derived apoptotic extracellular vesicles could promote wound healing and hair growth [67]. Jin et al. reported that calreticulin-mediated erythropoietic effects of MSC-derived apoVs were effective in the treatment of type 2 diabetes mellitus (T2DM) [68]. Therefore, further in-depth explorations of the isolation, identification, and biological functions of apoVs are required, along with elucidation of the properties of apoVs for their application in treatment of disease. Osteoporosis is a representative disease involving an imbalance between the formation of bone and adipose tissue. Our results showed that apoVs inhibited MSC adipogenesis, suggesting therapeutic potential in the treatment of osteoporosis as well as other aging-associated diseases. Furthermore, obesity is a risk factor for a wide range of diseases, including heart disease, cancer, androgenetic alopecia, and even infectious diseases such as COVID-19 [69–72]. Obesity has been shown to promote methylation of bone-bridge proteins leading to lipogenic differentiation of adipose-derived MSCs, and the ability of MSCs to differentiate into adipocytes is significantly higher in patients with T2DM than in healthy individuals, which may be one reason why these patients are prone to complications of obesity [73]. Therefore, investigating the mechanisms by which MSCs can be prevented from differentiating into adipocytes may be beneficial in the treatment of obesity and its complications.

Our understanding of the signaling mechanisms mediated by apoVs is still limited. Mouse bone marrow stem cell-derived apoptotic bodies repair host cell function by upregulating the host cell Wnt signaling pathway [18]. Epidermal stem cell-derived apoptotic bodies maintain epidermal tissue homeostasis by promoting the proliferation of neighboring cells through Wnt8a [74]. Apoptotic EVs activate the Fas pathway in multiple myeloma cells through Fas ligands to induce apoptosis and inhibit cell growth in multiple myeloma [66]. Here, we performed protein assays in apoV-treated and untreated control BMMSCs and showed that apoVs significantly affected the Wnt/β-catenin pathway. In subsequent studies, we will further explore the regulation of MSC adipogenesis by apoVs through the Wnt/β-catenin pathway *in vivo*, which will identify possible therapeutic targets and provide novel insights into the pathogenesis and treatment of osteoporosis and other clinical diseases related to the balance of osteogenesis and adipogenesis.

5. Conclusion

This study showed that BMMSC-derived apoVs reduced bone marrow adiposity via the Wnt/β-catenin signaling pathway, providing novel insight for the treatment of osteoporosis and obesity-related diseases, as well as other aging-related diseases.

Availability of data and materials

The authors confirm that all data underlying the findings are fully available.

Authors' contributions

YZ and KY were responsible for the conception and design, collection and/or assembly of data, data analyses and interpretation, and manuscript writing. YL, WW and YC were responsible for the collection and/or assembly of data and data analyses and interpretation in the animal experiments. RG, YD and HL were responsible for the collection and/or assembly of data and data

analyses in the molecular biology experiments. XZ and YL was responsible for the conception and design, financial support, and manuscript writing. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee of the Peking University Health Science Center (approval no. LA2023017). All surgeries were performed under anesthesia, and all efforts were made to minimize animal suffering.

Consent for publication

Not applicable.

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Declaration of competing interest

All authors disclosed no relevant relationships.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.reth.2025.03.012.

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