The balance between adipogenesis and osteogenesis in bone regeneration by platelet-rich plasma for age-related osteoporosis

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Abstract

The aim of this study was to develop a new diagnostic and therapeutic approach for the treatment of osteoporosis. Previously, we demonstrated that intraosseous transplantation of platelet-rich plasma (PRP) treated-osteoblast-like cells into ovariectomized senescence-accelerated mice (OVX-SAMP8) prevented the development of osteoporosis. In continuation, we aimed to explore the complex etiology of osteoporosis using this platform. An inverse relationship between bone marrow adipogenesis and osteogenesis has been suggested in the development of osteoporosis but the underlying mechanisms remain poorly described. To address these issues, we used PRP to inhibit adipocyte differentiation by promoting osteoblastic differentiation in bone marrow adipocytes. In addition, a positive correlation between an increase in bone marrow adipogenesis and osteoporosis has been observed. We assessed this relationship using an osteoporotic animal disease model which consisted of young (for prevention) and old (for treatment) OVX-SAMP8 mice. This animal model demonstrated that PRP treatment mainly exerted its action via promoting bone regeneration but also appeared to suppress adipogenesis within the marrow. The findings and methodology of this study could potentially be applied in the prevention and treatment of osteoporosis.

1. Introduction

Osteoporosis has become one of the most prevalent skeletal disorders in the developed countries. Osteoporosis is characterized by low bone mass and microarchitectural deterioration of the bones, leading to bone fragility and increased risks of fractures [1]. Osteoporosis is a complex skeletal disorder and clinically it is generally categorized into two broad types, the primary and secondary osteoporosis. Primary osteoporosis consists of two subtypes namely postmenopausal and senile osteoporosis [2]. Postmenopausal osteoporosis is the result of significant decreases in estrogen levels associated with menopause; while senile osteoporosis is mainly associated with aging. Secondary osteoporosis represents a specific collection of bone disorders which are associated with other medical conditions or as the result of certain therapeutic interventions [3,4].

Regardless of the etiology, osteoporosis reflects the disruption in bone remodeling process, in which bone resorption process proceeds more rapidly than bone formation. Thus, most currently available strategies used in treating osteoporosis involve agents which inhibit osteoblast activity hence bone resorption inhibition. Four major anti-resorptive agents used in clinics are estrogen, selective estrogen receptor modulators (SERMs), bisphosphonates and calcitonin [5–8]. Although these agents slow down the progression and severity of osteoporosis, serious side effects from long term usage are of clinical concerns. In addition, these agents cannot promote bone formation. Furthermore, as the population continues to age, osteoporosis is likely to become an even more prevalent and serious health condition with economic burden for the society. Therefore, more effective and safer therapeutic agents are urgently needed.
Bone remodeling is a complex process involving the close interactions between different progenitor cell lineages, mainly osteo- and adipo-progenitor cells. Clinically, it has been demonstrated that the increased adipogenesis and fat content in the bone marrow is correlated to the decreased bone mineral density in the elderly and osteoporotic population [9]. In mouse model, a decrease in the bone mineral density was accompanied by the formation of adipocytes and this phenomenon could be inhibited by the addition of 1,25(OH)2D3 [10]. These observations established an important link between osteo- and adipo-genesis. Since osteoblasts and adipocytes are both derived from mesenchymal stem cells within the bone marrow, signaling pathways involved in these two processes could be modified to favor osteogenesis and resulting in the amelioration of osteoporosis. It has been well demonstrated that PPAR-γ2 and c/EBP promote differentiation of adipocytes while RUNX2 and OSX promote differentiation of osteoblasts [11]. Previous studies have indicated that when normal osteogenesis is disrupted either under pathological conditions or clinical interventions, adipogenesis from mesenchymal stem cells took predominance. The increase in the number of adipocytes subsequently induced further apoptosis in osteoblasts and promoted the proliferation and differentiation of osteoclasts, resulting in an increase in bone resorption and overall bone loss [12,13]. This notion is supported by the clinical surveys indicating the long-term use of the drug rosiglitazone (Avandia) by diabetes patients lead to an increase of fat ratio and bone loss, which in turn increased the risks of osteoporosis. The study suggested that rosiglitazone promoted differentiation of adipocytes and lipotoxics, which in turn inhibited osteogenesis and lead to the development of osteoporosis [14,15].

Given the abundant observations that increased bone marrow fat content is closely associated with the development and/or consequence of osteoporosis, we intend to further explore the potential application of PRP for promoting osteogenesis of progenitor cells within the bone marrow using osteoporotic mouse model. We hypothesize that PRP could induce osteogenic differentiation in bone marrow progenitor cells including pre-adipocytes, enhancing osteogenesis and inhibiting adipogenesis in the marrow, thereby favoring bone formation and ameliorate osteoporosis. The goal of this study thus was to develop a method to stimulate bone formation in order to restore proper bone remodeling process. Our previous study has demonstrated that platelet-rich plasma (PRP) could stimulate the differentiation of embryonic fibroblasts into osteoblast-like cells and transplantation of these PRP-treated cells could significantly improve the bone architecture in O VX-SAMP8 osteoporotic mice [16]. We intended to explore the detailed mechanisms underlying PRP-mediated osteogenesis specifically in the alteration of marrow bone/fat ratio and its potential as a bone treatment for osteoporosis. The osteogenic effect of PRP was examined in two mouse groups: one designated the young mouse/preventative group, which consisted of one-month old mice that did not have osteoporosis; the other one assigned as the old mouse/treatment group which consisted of 10-month-old mice that already developed osteoporosis. This study contained two phases. In the first phase, we demonstrated the higher fat content in osteoporotic mice than that of their control littermates. In the second phase, PRP was used to demonstrate its ability to prevent and treat osteoporosis by controlling the ratio of osteoblast and adipocyte.

### 2. Materials and methods

#### 2.1. PRP preparation

Human total blood was purchased from Taipei Blood Center and separated out the PRP with MCS blood cell separation system (Haemonetics Corp., USA). The human PRP was prepared and stored at −20 °C. To verify the consistency and quality of purified PRP, the most abundant ingredient, transforming growth factorβ (TGF-β1) was quantitatively measured using a Quantikine enzyme-linked immunoassorbent assay (ELISA) kit (#DB100, R&D Diagnostics, Wiesbaden, Germany) and was used as a concentration calibrator for purified PRP. We have previously established that 750 pg/mL was optimal for cell proliferation and osteogenic differentiation [16].

#### 2.2. Cell culture and in vitro differentiation

Mouse pre-adipocytes (3T3-L1) and osteoblast cell line (7F2) (cat. no. CL-173 and CBL-12557) were purchased from the American type culture collection. 3T3-L1 cells were grown in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% CS and 1% PSA. When cells reached confluence (referred to day 0), the cells were cultured in adipocyte-differentiation medium (DMEM containing 0.5% IBS, 1 μM dexamethasone, and 10 μg/ml insulin) for 2 days and subsequently in fresh DMEM containing 10% FCS, 10 μg troglitazone and 10 μg/ml insulin every 2 days to obtain mature adipocytes. Co-culture experiments were performed under the following conditions. In the control group, adipocytes were co-cultured with osteoblasts (7F2) while in the experimental group, adipocytes were co-cultured with osteoblasts (7F2) in PRP-containing medium. Six well plates were used and the adipocytes (lower chamber) and osteoblasts (upper chamber) were separated by insert with polycarbonate membrane (0.4 μm) (Nalgene Nunc International, Naperville, IL) for 7 days.

To verify adipogenesis, cells were fixed with 10% formalin for 10 min, stained with 0.5% oil red 0 in 60% isopropyl alcohol for 15 min and rinsed with water. Oil red O stained lipid droplets were extracted by 100% absolute isopropanol and quantified by measuring absorbance in spectrophotometer at 540 nm. Osteogenesis was verified using Alizarin Red-S staining. Cells were fixed as described above followed by 2% Alizarin Red-S (pH 4.2) staining for 15 min at room temperature. For quantification, the bound staining was eluted with 10% cetylpyridinium chloride, and the absorbance of supernatants was measured at 540 nm.

#### 2.3. Experimental animals and PRP injection

The female SAMP8 mice experiment protocol was approved by the Institutional Animal Care and Use Committee of Taipei Medical University. The mice were maintained in the animal room under the temperature were be maintained at 25 °C with 50% relative humidity. Ovariectomized-SAMP8 female mice (1 month and 10 months of age) were used for experiments (each group contained 6 animals). Mice were then grouped into the follows: young control group (1 month-old, receiving PBS), young PRP (1 month-old animals receiving PRP treatment), old control group (10 month-old receiving PBS) and Old/PRP (10 month-old animals receiving PRP treatment). Both PBS and PRP were directly injected into the bone marrow cavity of the hind femur. After PRP injection, the animals were allowed to recover prior to further examination.

#### 2.4. Immunohistochemical analysis

To determine osteogenesis/adipogenesis in the treated mice, femurs and tibias from all groups were collected after 0 and 4 month of treatment. Bone samples were fixed in 10% formalin and processed. For morphological analysis, bone samples were first decalciﬁed in 14% EDTA for 3 days and subjected to parafﬁn-embedding. Bone sections (10 μm-tick) then were stained with H&E and observed under microscope for morphological analysis. To determine the degree of osteogenesis, OCT-embedded bone cryosections were used and without decalcification step. Sections were then subjected to Alizarin Red-S staining (calcium staining) and the intensity of the stain represented the degree of calcification within the sections observed.

#### 2.5. Bone mineral density (BMD) and micro-CT analysis

Dual-energy X-ray absorptiometry analysis (DEXA) analysis was established that measurement of bone mass in the spine, knee and femurs. The DXA (XR-36; Norland Corp.; host software revision 2.5.3, scanner software revision 2.0.0) was performed at time 0, 2, 3 and 4 months after different treatment with or without PRP. Bone samples from all groups were collected and imaged using a SkyScan-1076 Micro-CT System (SkyScan, Belgium). The following three-dimensional (3-D) parameters were measured: bone volume, total volume and trabecular bone numbers. For trabecular bone analysis and 3-D image construction, a micro-CT scanner (SkyScan-1076, SkyScan, Belgium) was operated at 50 kV, 200 μA, 0.4 μ of rotation step, 0.5 mm Al filter and 9 μm/pixel of scan resolution. The data collected was quantitatively represented as the percentage of bone volume/total volume and the number of trabecular bone (1/mm). Each group contained 6 animals.

#### 2.6. Isolation of bone marrow cells

We isolated the bone marrow cells from young and old mice with or without PRP treatment at 0 and 4 month. All femurs and tibia were cut into pieces and cultured in maintenance medium (x-minimum essential medium, 10% fetal bovine serum, and 1% prostate-specific antigens) for 1 wk. Non-adherent cells and bone pieces were removed and washed with PBS. The adherent cells, BMCs, were collected by trypsin and ethylenediaminetetraacetic acid treatment.

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2.7. RT-PCR and western blot analysis

All cells were harvested by scraping. Total RNA was extracts using TRIzol regents (Invitrogen Life Technologies). Gene expression levels were measured by RT-PCR. The PCR primers were as follow: BMP-2 forward primer 5’-GGTTTCTTCAACAACAGATGAAC-3; reverse primer 5’-CAACCTCCACACCATGTT-3; annealing temperature 62 °C, Osteopontin(OPN)-forward primer 5’-ATGAGATGGCGAGTTGAT-3; reverse primer 5’-GTCGTCCACTCAGATGAAGT-3; temperature, 48.8 °C, Peroxisome proliferator-activated receptor gamma2 (PPARγ2) forward primer 5’-CATTCACAGAAATACCAT-3; reverse primer 5’-GA.

GGACCTTATGTTAGCTGATC-3; temperature 62 °C, Leptin-forward 5’-TGTCAGAAGATGGACATTGAC; reverse primer 5’-GAGTAGATGAGGCTTCCAGGA-3; temperature 62 °C; Runt-related transcription factor 2 (RUNX2) forward primer 5’-ACTTCCTCGAGAACAGTGC-3; reverse primer 5’-GCTTTGTCCTCCTGCTGATC-3; temperature 55 °C; Osteocalcin (OCN) forward primer; 5’-CAGCTTGGTGCAACCTCAAGC-3; reverse primer 5’-AGGGTTAAGCTCACACTGCTC-3; temperature 55 °C; Receptor Activator of Nuclear factor kappa B (RANK) forward primer 5’-TCAGGTCATCCTCTCAGTC-3; reverse primer 5’-GTCAGCTGTCGACACGAGC-3; temperature 60 °C; Receptor Activator of Nuclear factor kappa B ligand (RANKL) forward primer 5’-ATCACGAGCACAGATCTCAC-3; reverse primer 5’-TGGTCTGTCTCCTCTGTTAC-3; temperature 62 °C; Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control (CTRL) forward primer 5’-GCTTCACAAGAATCCTCAGGA-3; reverse primer 5’-GCTTTGTCCTCCTGCTGATC-3; temperature, 55 °C; PCR products were separated by electrophoresis on 1% agarose gels (Aagarose l; AMRESCO) and visualized with ethidium bromide staining. Western blot analysis was performed according to a standard protocol. All cells were lysed in RIPA buffer (Thermo scientific) and separated by SDS-polyacrylamide gel electrophoresis. The primary antibodies used were purchased from Novus biological (OPN, BMPR-IB, Beta-Actin (CTRL) and RUNX2), Santa Cruz biotechnology, Inc. (PPAR-γ), and Abgent primary antibody company (BMPR-IA). Respective secondary antibody were used and immunoperoxidase reactions were performed by SuperSignal West Pico Chemiluminescent substrate. (Biolyx Inc.)

2.8. Statistical analysis

All results were represented as mean ± standard deviation (S.D.) Significant differences between two groups were determined by student’s t test, p value < 0.05.

3. Results

3.1. PRP-induced osteogenesis in pre-adipocytes, 3T3-L1 cells

To determine PRP’s effects on promoting osteogenesis, an established pre-adipocyte cell line 3T3-L1 was used as a cell model. 3T3-L1 cells were cultured in adipogenesis-promoting medium (AM) with or without PRP for 7 days. Without the presence of PRP (APM only), a significantly up-regulation in osteogenic markers such as PPAR-γ2 and leptin accompanied with a marked down-regulation in osteogenesis markers including RUNX2, OPN and OCN, was detected (Fig. 1A). In contrast, the reversed was observed in PRP-treated 3T3-L1 cells, especially for up-regulation in osteogenesis. These observations were further validated by the biochemical analysis where 3T3-L1 cells exhibited an increased level of cytoplasmic lipid droplet deposition while PRP-treated counterparts showed an elevated level of matrix mineralization (Fig. 1B). These results indicated that the presence of PRP promoted pre-adipocyte towards osteogenesis rather than adipogenesis.

3.2. PRP induced osteogenesis in matured adipocytes

After establishing that PRP could promote osteogenesis in pre-adipocytes, we examined if PRP could exert similar effects in differentiated adipocytes. Pre-adipocytes were first differentiated to adipocytes (induced-adipocytes) in adipogenesis-promoting medium for 7 days followed by another week of incubation in PRP-containing medium. We found that both PPAR-γ2 and leptin expression were significantly decrease in the adipocytes receiving PRP treatment. In addition, adipocytes cultured in PRP-containing medium exhibited an increased expression levels in genes involved in osteogenesis such as RUNX2, OPN and OCN (Fig. 2A). Similarly, high degree of bone matrix mineralization was observed in PRP-treated adipocytes but was non-detectable in control adipocytes (Fig. 2B). These results indicated that PRP could promote transdifferentiation of differentiated adipocytes into osteogenesis.

3.3. Transdifferentiation of adipocytes by PRP via activation of BMP signaling

Dynamic cellular communications occur among the progenitor cells participating in osteogenesis and adipogenesis. These interactions play key roles in determining the final cellular fate for these progenitor cells, towards either osteogenesis or adipogenesis [17].

![Fig. 1](https://example.com/fig1.png)

Fig. 1. Osteogenesis-promoting effects of PRP in 3T3-L1 progenitor cells. (A) PRP-treated 3T3-L1 cells demonstrated a significant down-regulation of adipogenic markers such as PPAR-γ2 and Leptin while an up-regulation of osteogenic markers including RUNX2, OPN and OCN, at both transcriptional and translational levels. In contrast, 3T3-L1 cells treated with adipogenesis-promoting medium (AM) alone did not show significant enhancement of osteogenic markers as observed in the PRP-treated group (B) Lipid droplet formation was markedly hampered in PRP-treated 3T3-L1 cells as shown by decreased oil red O staining (lower left panels) whereas calcium staining (lower right panels) was significantly increased, suggesting PRP treatment promoted osteogenesis in 3T3-L1 progenitor cells. Upper panels indicate the quantitative representations of staining intensities observed. All experiments were performed in triplicates. *: P < 0.01 vs other groups.
To better recapitulate the dynamic interactions between osteogenesis and adipogenesis within the bone marrow microenvironment, 3T3-L1 and 7F2 were co-cultured under either adipogenic conditions and/or the addition of PRP. To this end, we set out to examine the role of PRP in promoting osteogenesis in an osteoblasts/adipocytes co-culture system. The proliferation rate of osteoblasts was significantly decreased after co-culturing with adipocytes, and this decrease of proliferation rate was then significantly recovered in the presence of PRP (Fig. 3A, column 3,4 and 5). The proliferation rate of adipocytes co-cultured with osteoblasts appeared to be unaffected. Next, we examined the underlying molecular mechanisms involved in PRP-induced osteogenesis. BMP signaling pathways have been shown to regulate osteogenesis/adipogenesis. BMPR-IB promotes osteoblast differentiation whereas BMPR-IA induces adipocyte differentiation [18]. In PRP-treated pre-adipocytes, the expression and activity of BMPR-IB and BMP-2 were significantly up-regulated when compared to their counterparts without PRP. In addition, PRP treatment reduced BMPR-IA expression in pre-adipocytes (Fig. 3B and C). These findings collectively indicated that PRP promoted osteogenesis by up-regulating BMP-2 and BMPR-IB and suppressing BMPR-IA pathways (Fig. 3D).

### 3.4. In vivo demonstration of inverse relationship between bone marrow fat content and bone mineral density

To demonstrate the inverse relationship between marrow fat content and bone formation, we used previously established osteoporotic O VX-SAMP8 mice as our model [16]. Both trabecular bone mass and volume in old (10 month of age) O VX-SAMP8 mice were significantly lower than those in young (1 month of age) as observed in micro-CT (2-D and 3-D) images. Histological analysis of bone tissues from old O VX-SAMP8 animals revealed an increased level of adiposity as compared to that of in the young mice (Fig. 4A). Quantitative data obtained from the images was shown in the lower panel. Subsequently, we isolated and analyzed the bone marrow cells in gene and protein level from young and old mice. Adipogenesis specific marker, PPAR-γ2 was found to be up-regulated while osteogenesis specific markers such as RUNX2 and OPN were down-regulated in osteoporotic old mice, but not in the young counterparts (Fig. 4B). These observations demonstrated the reciprocal relationship between bone marrow fat content and osteogenesis.

#### 3.5. Improvement of bone mineral density (BMD) in PRP-treated osteoporotic O VX-SAMP mice

The bone mineral density (BMD) score was used as a key to assess the bone sample quality in both young (Fig. 5A) and old (Fig. 5B) O VX-SAMP8 mice. A strong trend of improved BMD score was observed in bone samples from the spine, both knee joints and femurs in RPR-treated animals after 4 months. Importantly, PRP treatment prevented the BMD from decreasing in young O VX-SAMP8 (Fig. 5A). In addition, in the osteoporotic old O VX-SAMP8 animals, RPR treatment appeared to significantly improve the BMD scores (Fig. 5B).

The improvement in BMD scores in PRP-treated animals was further supported by bone morphological analysis. Micro-CT 2-D and 3-D imaging both demonstrated higher trabecular area and volume in both young and old RPR-treated O VX-SAMP8 animals (first two columns respectively, Fig. 6A). Consistently, histological sections of the bone samples from PRP-treated mice revealed more trabecular bone areas and more intense calcium staining (last two columns respectively, Fig. 6A). When the relative trabecular bone volume ratio to the total bone volume was measured, it was clear that both PRP-treated mice, young and old, demonstrated a significantly higher trabecular bone ratio (upper panel, Fig. 6B); a higher trabecular bone number was also found in both PRP-treated mice, young and old (lower panel, Fig. 6B).

#### 3.6. The treatment of PRP induces in vivo osteogenesis and suppresses adipogenesis in O VX-SAMP animals

Bone marrow cells were isolated from control and PRP-treated O VX-SAMP8 animals to determine signaling pathways involved in PRP-mediated osteogenesis. Both transcriptional (left panel, Fig. 6C) and translational (right panel, Fig. 6C) levels of adipocyte differentiation (PPAR-γ2) and osteoblast differentiation (RUNX2 and OPN)
Fig. 3. Dual role of BMP signaling pathway on adipocyte and osteoblast differentiation of 3T3-L1 cells by PRP. When co-cultured with adipocytes, osteoblast proliferation was inhibited but was resumed by the addition of PRP treatment after 7 days. (A). Western blot analysis demonstrated the dynamic expression profile for BMP-2 and its receptor (BMPR) isoform IB and IA of 3T3-L1 cells treated with PRP. Interaction with BMPR-IB is generally osteogenic, while that with BMPR-IA is adipogenic. BMP-2 and BMPR-IB expression level in PRP-treated 3T3-L1 cells was increased while BMPR-IA was decreased (B and C). PRP promoted osteogenic differentiation of adipocyte via BMP-2 binding with high affinity to the type IB receptors pathway (D). *: P < 0.01 vs osteoblast group. #: P < 0.01 vs co-osteoblast group.

Fig. 4. Age-related bone loss and adipogenesis in SAM mice. Micro-CT and H&E staining comparative analyses of bone structure between young and old OVX-SAMP8 mice. Micro-CT (2-D and 3-D) imaging showed a significant decrease in trabecular bone mass in the old animals when compared to the young mice. A higher ratio of bone marrow adiposity (arrowheads point to the adipocytes) was observed in the old mice than that of in the young mice (A). The lower panel represented quantitative data obtained from the images (n = 6). The expression level of adipogenic marker (PPAR-γ2) appeared to increase in the aged SAMP8 mice whereas both osteogenic markers (RUNX and OPN) showed a reduction, as demonstrated by RT-PCR and western blot analysis respectively (B). E*: P < 0.01 vs Old group.
markers were evaluated. PPAR-γ2 expression level was down-regulated while RUNX2 and OPN were significantly increased in PRP-treated mice when compared to respective control mice. These findings suggested that PRP treatment resulted in suppressing adipogenesis and promoting osteogenesis in osteoporotic mice.

In addition, when we compared the molecular difference between the bone formation (OPN) and resorption (RANKL) processes in both young and aged mice, we found that bone forming process marker OPN in young was higher than the aged counterparts while reversed was observed for the bone resorption process marker RANKL (Supplementary Fig. 1A). After the addition of PRP in both young and aged mice for 4 months, it appeared to suppress bone resorption process, as evident by the decreased RANKL transcript (Supplementary Fig. 1B). Collectively, PRP-mediated osteogenesis was the result of favoring bone formation process over resorption.

4. Discussion

Recent studies have suggested important connections between the bone remodeling process and marrow fat contents as well as the implications towards the etiology of osteoporosis. These findings indicated that osteoporosis could be the result of age-related infiltration of marrow fat which suppressed osteoblast differentiation, function and survival [9,19]. Based on these findings, we hypothesized that bone marrow fat content could be modulated and used as a diagnostic and therapeutic approach for osteoporosis. We attest this hypothesis using plate-rich plasma (PRP) as a molecular cocktail to modulate the extent of adipogenesis and osteogenesis in vitro and in osteoporotic mouse model.

PRP was chosen as the molecular modulator of osteogenesis because it contains a rich spectrum of growth factors including high concentrations of PGDF, TGF-β1, IGF and so forth. Thus PRP has been widely used in various clinical procedures, specifically in the promotion of cellular growth [20]. More importantly, data from others and our laboratory have indicated PRP’s ability in promoting osteogenesis [21]. Specifically, PDGF has been shown to promote the proliferation and differentiation of osteoblasts while TGF-β1 inhibits the differentiation of adipocytes but enhances the proliferation and differentiation of osteoblast. IGF plays important roles in bone matrix synthesis and fat elimination [22–26]. Collectively, PRP represents an ideal molecular modulator for osteogenesis.

In this study, we provided evidence that the addition of PRP did not promote the maturation of pre-adipocytes (3T3-L1) into adipocyte. More importantly, PRP not only inhibited adipogenesis of pre-adipocytes but also promoted osteogenesis. PRP-induced osteogenesis was achieved by simultaneously up-regulating osteogenesis-promoting genes RUNX2, OPN and OCN while downregulating adipogenesis regulators such as PPAR-γ2 and leptin. In addition, PRP treatment enhanced BMP-2 and BMPR-IB and suppressed BMPR-IA pathways in pre-adipocytes. This is in agreement with the opposing roles of BMP-2/BMPR-IB and BMPR-IA in osteogenesis [27]. Collectively, we demonstrated the trans-differentiation of adipocytes to osteoblasts without genetic manipulation which involved major gene knockout, PPAR-γ [28,29].

To examine the potential usage of PRP in treating osteoporosis, we used a previously established mouse model, OVX-SAMP8 which simulates senile osteoporosis in human. Using this system, we were able to establish key features for the study of osteoporosis. First, we created a standardized system for examining osteoporosis using BMD scan and morphological validation (immunohistochemistry and scanning electron microscopy) so that the degree of osteoporosis could be monitored and validated consistently and accurately [16]. Second, in this study, we added another key feature, marrow fat content, which could be used not only for diagnosis but also a therapeutic assessment. In agreement with others, our histological
analysis of the bone sections from old OVX-SAMP8 mice revealed a significantly lower ratio of osteogenesis over adipogenesis. Coincidentally, a lower BMD score was also detected in these old mice exhibiting a higher adipose tissue content in the bone marrow. These features combined, OVX-SAMP8 represents an ideal animal system for studying human osteoporosis [10,30].

Osteogenesis-promoting ability of PRP was investigated in vivo using OVX-SAMP8 mice exhibiting low BMD scores and coincidently high marrow fat contents. We found that the BMD scores were higher in the PRP-treated OVX-SAMP8 mice as compared to their counterparts. In addition, PRP treatment appeared to prevent the occurrence of osteoporosis in the young OVX-SAMP8 animals. These data reinforced the notion that PRP treatment induced osteogenesis and the marrow fat content could potentially be used as a diagnostic and therapeutic marker for osteoporosis.

Enhanced osteogenesis by PRP treatment was accomplished via the simultaneous up-regulation of osteogenesis and down-regulation of adipogenesis. Bone marrow cells obtained from PRP-treated animals demonstrated markedly elevation in the expression of osteogenesis-related molecules such as RUNX2 and OPN and suppression of major adipogenesis regulator PPAR-γ2 and RANKL. The ratio of RANKL/OPG was used as an indicator for osteoclastogenesis. In the young animals, the secretion of RANKL was lower and the expression of OPG was higher than that of old mice. This data indicated that old osteoporotic mice exhibited a significantly higher level of bone resorption leading to bone loss. Bone marrow cells from osteoporotic mice following PRP treatment demonstrated an increased expression of OPG and decreased level of RANKL, as an indication of osteoclastogenesis suppression. Collectively, these findings were in accordance to our in vitro data that PRP promoted osteogenesis over adipogenesis.

Due to the pluripotent characteristics of stem cells (either of embryonic or adult bone marrow origin); stem cell-based therapies have attracted researchers and clinicians in treating a variety of different disorders including osteoporosis. A number of studies have demonstrated the sole use of cell therapy, or cell therapy in conjunction with transgenic techniques to drive and promote bone regeneration. However, one of the major concerns for these well-orchestrated methods is the potential tumorigenic/teratogenic effects from the transplanted stem cells and/or genetic modifications [31–33]. In addition, obtaining and maintaining transplanting cells at specific differentiation stage remains a major technical hindrance. For these reasons, current study avoided the usage of stem cells and demonstrated that marrow-injected PRP alone could achieve a significant level of osteogenesis in treating and preventing osteoporosis in experimental animal model. As an important safety consideration, PRP-treated animals exhibited no detectable signs of tumorigenesis over the course of study.

Osteoporosis has been recently suggested as a disorder of lipotoxicity. This term adds a new perspective that bone loss is
accompanied or as a consequence of a high percentage of marrow fat. It has been shown diabetic patients who exercise long term usage of thiazolidinediones (TZD) have a greater risk of bone fractures and osteoporosis [14,15]. This could be attributed to the fact that TZD drugs are potent fat agonists and adipogenesis promoting agents, which eventually lead to the imbalance in bone remodeling process and bone loss. Co-culturing adipocytes with osteoblasts shows that adipocytes negatively influenced the proliferative capacity of osteoblast [12]. Studies also suggested that increasing the PPAR activity of adipocytes led to high ROS accumulation and caused eventual apoptosis of osteoblasts [34,35]. We also showed that transplanting a large number of adipocyte into the bone marrow of mice caused bone loss (our unpublished data). Our in vitro data showed that adding PRP to the co-culture of adipocyte and osteoblast could prevent the proliferation of osteoblast from being inhibited by adipocyte; in vivo, we showed that PRP treatment enhanced osteogenesis while reduced marrow adipogenesis. Future research could be conducted to investigate whether PRP can control oxidative stress, mitochondrial dysfunction, and apoptosis.

5. Conclusions

In this study, we developed an experimental platform for studying osteoporosis and a potential strategy for the treatment/prevention of this disorder. PRP treatment was shown to improve overall bone quality in osteoporotic mice via promoting osteogenesis while suppressing adipogenesis in the bone marrow. Therefore, our study provided experimental support for the potential application of PRP for treating osteoporosis in the clinical settings. Additionally, a diagnostic system based on the ratio between marrow adipose contents and bone density could be used for the assessment of osteoporosis.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biomaterials.2011.05.080

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