Reduced Anabolic Response To Parathyroid Hormone In Periosteal Mesenchymal Stem Cells From Aged Mice

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Introduction: Parathyroid hormone (PTH) 1-34 is approved for osteoporosis treatment, and stimulates fracture healing [1]. Intermittent PTH 1-34 treatment increases bone formation and biomechanical strength of fractures [1]. We have shown that fracture repair is delayed in aged mice [2], whereas PTH 1-34 enhances periosteal progenitor cell proliferation from both young and aged mice [3]. It is unknown if the decreased reparative potential observed in aged mice is due to a decrease in the osteogenic capacity of periosteal mesenchymal stem cells (MSCs), and if altered PTH signaling is involved in this process. We hypothesize that reduced responsiveness to PTH 1-34 in periosteal MSCs contributes to the impaired bone regenerative capacity in aged mice.

Methods: A 4 millimeter mid-diaphyseal segment was removed from the femur and fresh bone graft was immediately transplanted into the same mouse. Periosteal MSCs were isolated from the surface of the femoral autografts of young (2 months old) and aged (15 months old) C57BL/6J female mice at day 5 post-transplantation. Bone marrow was removed by repeatedly flushing of the marrow cavities with serum free alpha-MEM medium. Cells from young and aged mice were cultured in MesenCult™ MSC basal medium (mouse) supplemented with the MesenCult™ MSC stimulatory supplements (mouse) from STEMCELL Technologies Inc. Flow cytometry data showed that more than 90% of the first passage cells expressed surface markers of MSCs (CD29+, CD105+ and Sca1+), but were negative for CD45 and CD31. The cells collected from second and third passage were cultured at the density of 2×10^3/cm^2. When the periosteal MSCs were grown to 80% confluence, the culture medium was changed to an osteoblast inducing medium containing10 mM beta-glycerophosphate, and 0.05 mM L-ascorbic acid. The differentiated cells were treated with PTH 1-34 (10 nM) every two days. Alkaline phosphatase (ALP) and alizarin red staining, osteoblast gene expression (i.e., Col1a1, ALP and osteocalcin) using real-time RT-PCR analysis, and colony-forming unit osteoblasts (CFU-OB) assay were performed.

Results: Periosteal MSCs from aged mice had lower osteogenic differentiation capacity than those from young mice (Figure 1 and 2). CFU-OB assay demonstrated that the clonogenic ability of periosteal MSCs from aged mice was decreased as compared to that from young mice (Figure 3). Reduced osteogenic differentiation was also observed after PTH 1-34 (10 nM) treatment in periosteal MSCs from aged mice relative to young mice (Figure 1). PTH 1-34 (10 nM) treatment increased the clonogenic ability of periosteal MSCs from both young and aged mice (Figure 3). Western blot showed that decreased level of PTH1 receptor was observed in periosteal MSCs from aged mice as compared to young mice (Figure 3). In conclusion, periosteal MSCs from aged mice exhibit a reduced regenerative capacity during bone healing, which is due in part to decreased PTH signaling and PTH1 receptor expression.

Discussion: MSCs can secrete specific osteogenic factors during injury to drive the bone repair process. A critical step in bone repair is the proliferation of periosteal MSCs, which then undergo osteoblast and chondrocyte differentiation, and stimulate re-vascularization of the healing site [4]. PTH 1-34 is reported
to increase fracture strength, callus volume and callus bone mineral content in 27-month-old rats [5]. This may be due to the increased differentiation of MSCs into osteoblasts [6]. This is corroborated by our previous findings showing that PTH 1-34 enhances periosteal progenitor cell proliferation in both young and aged mice [3]. These observations suggest that the bone anabolic effects of PTH 1-34 are the results of an improved periosteal response during fracture repair. It has been shown that aging impairs bone healing [7]. In the present study, we have shown that periosteal MSCs from aged mice exhibited decreased regenerative capacity and anabolic response to PTH 1-34 as compared to that from young mice. PTH induces MSC differentiation to the osteoblast lineage by endocytosis of the PTH1 receptor/ lipoprotein receptor-related protein 6 complex, allowing enhancement of bone morphogenetic protein signaling [6]. We observed that PTH1 receptor levels were reduced in periosteal MSCs from aged mice compared to those from young mice. Hence, the reduced regenerative capacity of periosteal MSCs from aged mice is due at least in part to PTH1 receptor expression. Further experiments using PTH1 receptor knockout mice will further reveal the role of PTH/PTH1 receptor signal in fracture healing in vivo.

Significance: Although PTH 1-34 has been used in human clinic trials to enhance fracture healing [8], the mechanism of this bone anabolic effect is unclear. This study showed that periosteal MSCs are the targets of PTH 1-34, and PTH1 receptor reduction is associated with the reduced efficacy of PTH 1-34 in periosteal MSCs from aged mice during bone healing.

![Figure 1. Representative images for Alizarin red (A) and ALP (B) staining. PTH increases osteogenic differentiation in periosteal MSCs from young and aged mice.](image-url)
Figure 2. Osteoblast gene expression. (A) Col1a1, (B) ALP, and (C) Osteocalcin. Osteoblast gene expression is lower in periosteal MSCs from aged mice than that in periosteal MSCs from young mice. *p<0.05 and **p<0.01 compare to young control group at day 0; #p<0.05 and ##p<0.01 compare to aged control group at day 0; &p<0.05 compare to young group at day 14 (N=3).

Figure 3. Representative images of Western blot (A) and CFU-OB (B) assay in periosteal MSCs from young and aged mice. Reduced level of PTH1 receptor is observed in periosteal MSCs from aged mice as compared to young mice. Moreover, PTH 1-34 (10 nM) treatment increases the clonogenic ability of periosteal MSCs from both young and aged mice.

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