MUSCLE-DERIVED CELL-MEDIATED BMP4 DELIVERY IMPROVES BONE FORMATION DURING EARLY PHASE OF OSTEOPOROTIC FRACTURE HEALING

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Introduction:
Recent studies have shown impaired fracture callus healing in the osteoporotic bones of ovariectomized (OVX) rats [1-2]. Muscle cell-mediated gene therapy to deliver BMP4 has successfully enhanced bone formation at fracture site in normal animals [3]. We hypothesized that osteoporotic fracture healing also might be improved via the implantation of muscle-derived cells (MDCs) genetically engineered to express BMP4.

Method:
Sixty 10-week-old female Fisher 344 rats were used for this experiment. Forty rats underwent bilateral ovariectomy. The remaining 20 rats were used as a normal control group (NC). Three months after surgery, the development of osteoporosis in the OVX rats was validated by body weight measurement and assessment of the quality and quantity of cancellous bone in the tibial head by micro-CT (μ-CT200, Scanco Medical, Bassersdorf, Switzerland). Primary MDCs were isolated from a normal rat and prepared for transplantation as described previously [3]. A unilateral mid-diaphyseal femur osteotomy was created after the fracture site was stabilized with a custom-made polyethylene plate and 4 K-wires. The OVX rats were divided into two treatment groups: an osteoporotic control group (OC), in which animals were treated with LacZ-expressing MDCs, and an osteoporotic treatment group (OT), in which animals were treated with BMP4-expressing MDCs. Animals in the normal control group (NC) received no cell treatment. Fracture healing was monitored by histology (hematoxylin and eosin stain) and micro-CT at 4 and 12 weeks post-surgery. The bone volume (BV; the total amount of newly formed bone) at the osteotomy site and other histomorphometric parameters (trabecular number, thickness, and separation) were calculated and compared statistically.

Results:
Gains in body weight and changes in bone micro-structural parameters in the proximal tibia confirmed the development of osteoporosis in rats after ovariectomy. Histological evaluation revealed delayed endochondral ossification at the fracture site in the osteoporotic control group (OC). Enhancement of new bone formation to a level similar to normal bone was obvious in the MDC-BMP4 treatment group by 4 weeks post-surgery. A significantly lower BV and trabecular number (p<0.05) was observed in the OC group (1.1 mm³ and 1.7/μm) when compared to the NC group (2.7 mm³ and 2.4/μm) at the 4-week time point. A significantly higher BV and trabecular number (3.0 mm³ and 2.8/μm) was observed in the OT group when compared to the OC group (Figure 1). No difference in trabecular thickness or separation was detected at the 4-week time point. At 12-weeks post-surgery, no significant differences in BV or other histomorphometric parameters were detected between any of the groups (Figure 2).

Discussion:
Age and ovariectomy have been shown to significantly reduce bone mineral density (BMD) in intact bones and impair gain in BMD in fracture-treated bones [1]. In the present study we also observed delayed ossification and significant decreases in new bone volume during osteoporotic fracture healing.

The use of gene therapy to deliver BMPs may provide the constant source of therapeutic protein required for the enhancement of bone formation in osteoporotic bones that exhibit a reduced healing capacity. Indeed, we observed early improvements in fracture callus formation in the group treated with MDCs expressing BMP4. More importantly, the new bone volume and number of trabeculae at the treated osteoporotic fracture site was comparable to that in normal rats.

These findings may have important implications for the development of future treatment options for osteoporotic fractures.

References:

Figure 1. The volume of newly formed callus at the osteotomy site after 4 weeks.

Figure 2. The volume of newly formed callus at the osteotomy site after 12 weeks.

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