Age-related Differences in BMP-2-mediated Bone Repair

Albert Cheng¹, Laxminarayanan Krishnan¹, Lisa Tran², Robert E. Guldberg¹.
¹Georgia Institute of Technology, Atlanta, GA, USA, ²Emory University School of Medicine, Atlanta, GA, USA.

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Introduction: The repair of large bone defects is one of the most challenging problems faced by orthopaedic surgeons today. Current treatments involve bone grafts and/or delivery of osteoinductive proteins such as bone morphogenetic protein 2 (BMP-2). However, the use of BMPs, especially at the high doses used clinically, is associated with increased inflammation, ectopic bone formation, and osteolysis. Furthermore, BMP-2 is currently contraindicated by the FDA for use in pediatric patients due to reported cases of massive inflammatory reactions and because the BMP dose calculations for children has yet to be defined. Despite this warning, BMPs are still commonly used in pediatric patients to repair large bone defects resulting from injury, tumor resection, or congenital deformity. The overall goal of this project is to establish a pre-clinical small animal model using adolescent rats to characterize bone regeneration in the pediatric population. Our group has previously established a critically-sized segmental bone defect model in adult rats and determined the minimum healing BMP-2 dose for delivery in a collagen sponge, which is the current clinical standard. The objective of this study was to extend our model to adolescent rats and evaluate differences in the BMP-2-mediated healing response, particularly at the early healing stage when inflammation is likely to be a key player in the pathophysiology of bone healing. We hypothesized that compared to mature rats, the adolescent rats would show higher expression of genes related to bone healing at lower BMP-2 doses and also exhibit an increased inflammatory response at higher BMP-2 doses.

Methods: All procedures were reviewed and approved by the Georgia Tech IACUC. 7-week-old and 8-month-old male Sprague-Dawley rats received bilateral surgeries to create 8 mm mid-femoral bone defects. A cylindrical collagen sponge (5mm in diameter) was loaded with 150 μl of BMP-2 solution and then placed in the bone defect. Each rat received two doses of BMP-2: a low 1 μg and a high 10 μg dose (n=4). Unoperated rats in each age group (n=2-3) were used as controls for gene expression analysis. After 1 week, all animals were euthanized and tissue in the bone defect and the surrounding musculature were collected and stored in RNAlater (Qiagen) at 4°C. RNA isolations were performed by following a standard Qiazol extraction protocol, and then cDNA was synthesized using RT2 First Strand kits (SABiosciences). Finally, qRT-PCR was accomplished using the Fluidigm Biomark system to assess the expression of 48 genes. Fold change was calculated after normalization to the geometric mean of 5 housekeeping genes. Analysis was performed using JMP Genomics software and two-way ANOVA was used to determine significance.

Results: For ease of analysis, the 48 genes were divided into seven groups related to cell recruitment, osteogenesis, angiogenesis, myogenesis, chondrogenesis, inflammation, and housekeeping. Preliminary gene expression analysis revealed interesting trends amongst the groups. Using hierarchical clustering of the normalized gene expression data, we saw that the young 7-week-old animals exhibited higher expression of osteogenic genes both inside the bone defect (Fig. 1) and in the surrounding muscle (not shown). In contrast, the mature 8-month-old animals demonstrated higher expression of inflammatory
genes in bone defect (Fig. 2) and muscle tissues (not shown). Interestingly, the gene expression patterns were clustered by age of the animals rather than the dose of BMP-2. Within the age groups, the younger animals qualitatively showed increased expression of both osteogenic (OPN, OSX) and osteoclastic (OPG, RANKL) genes at the higher BMP-2 dose (Fig. 1). Meanwhile in the older animals, the pro-inflammatory genes (IL-1A/B, TNF) were qualitatively upregulated at the higher BMP-2 dose (Fig. 2). Statistical analysis revealed differences between treated groups and unoperated controls, but only a few genes exhibited differences between age groups receiving the same BMP-2 treatment. Two such genes were PAX7 and MYH2 (Fig. 3), which encode for different proteins critical to proper muscle cell maturation and function. However, several genes demonstrated signs of possible differential regulation with BMP-2 dose and age that may become significant with more forthcoming data.

**Discussion:** In partial support of our hypothesis, the adolescent animals showed higher expression of most osteogenic genes in the healing defect at both BMP-2 doses, suggestive of a greater healing response even at this early time point. Contrary to our hypothesis, the mature animals exhibited higher overall expression of inflammatory genes. It is possible that the younger animals have resolved the majority of acute inflammation by 1 week and have shifted into a matrix synthesis stage more quickly, which would further support the previous claim that the adolescent animals possess superior healing capacity. In addition, the muscle repair response appeared blunted in the older animals based on PAX7 and MYH2 expression. We expect additional significant differences in gene expression profiles to be revealed once we complete analysis of the full study of samples (n=6-9 per group).

**Significance:** Age-related differences in the dose response to BMP-2 have yet to be characterized. Our study to assess differences in bone repair between adolescent and mature rats may reveal important strategies for optimizing treatments for different patient populations.

![Figure 1: Hierarchical clustering of osteogenic gene expression at 1 week in bone defect tissue](image-url)
**Figure 2:** Hierarchical clustering of inflammatory gene expression at 1 week in bone defect tissue.

**Figure 3:** Sample plots of (A) osteogenic, (B) myogenic, (C) angiogenic, and (D) inflammatory gene expression in muscle.

* p < 0.05 vs. 7 week old control. # p < 0.05 vs. 8 month old control.