Enhancement of Bone Regeneration through Apoptosis Modulation

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Approximately 16 million bone fractures are reported each year in the Unites States, with 10-15% of these fractures complicated by delayed healing or nonunion. Delayed and nonunions result in prolonged pain and disability that significantly impairs quality of life, productivity, and income for these patients. For example, it is estimated that while a single tibial nonunion fracture accrues direct medical costs of only ~$7,500, this amount is dwarfed by the approximately $17,000 in associated costs that are incurred from the indirect effects (i.e., work absenteeism, child care, travel) that accompany these cases (Busse et al., 2005). Therefore, the development of new therapeutics that enhance bone regeneration will not only result in reductions is pain and disability, but also significant monetary savings. We have been working for several years to elucidate the molecular basis of fracture repair, as we believe that the identification and characterization of critical regulatory pathways in bone regeneration is vital to the development of new therapeutics to enhance this process.

One such series of studies assessed the role of HIF-1 alpha in bone regeneration. The results revealed that partial HIF-1 alpha deficiency enhances bone regeneration, with fracture calluses from HIF-1 alpha+/- mice exhibiting greater mineralization and mechanical superiority to those from wild-type littermates. Subsequent molecular and cellular analyses identified the mechanism underlying this enhancement in bone regeneration as reduced apoptosis in osteoblasts and chondrocytes. We now seek to further characterize the process of apoptosis during bone regeneration and directly assess the efficacy of apoptosis inhibition in enhancing bone regeneration. These studies will test our central hypothesis that apoptosis is a critical regulatory process during bone regeneration; and its inhibition will enhance bone regeneration. The proposed experiments will be performed using a well-established murine closed femoral fracture model of bone regeneration and utilize a range of outcomes measures from the molecular to the organ level in the completion of the following two Specific Aims:

Specific Aim 1: Characterize the process of apoptosis during bone regeneration. We hypothesize that specific apoptotic signaling pathway components are activated during bone regeneration. We will test this hypothesis by generating closed femoral fractures in mice and (A) assessing the spatiotemporal levels of osteoblast and chondrocyte apoptosis using Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and osteoclastogenesis using tartrate-resistant acidic phosphatase (TRAP) staining throughout the stages of the regenerative process and (B) identifying activated apoptotic signaling pathways components using RNAseq, quantitative polymerase chain reaction (qPCR), immunoblots, histology, and immunohistochemistry (IHC).

Specific Aim 2: Validate the efficacy of pharmaceutical apoptosis inhibition to enhance bone regeneration. We hypothesize that inhibition of apoptosis will enhance bone regeneration after fracture. We will test this hypothesis by performing closed femoral fractures in mice, treating them with vehicle or BAF (an apoptosis inhibitor), and (A) confirming apoptosis inhibition using TUNEL staining, RNAseq, qPCR, immunoblots, histology, and immunohistochemistry, as well as (B) determining changes in functional bone regeneration using microCT and biomechanical testing.

The results from these experiments will provide the first comprehensive explication of the apoptosis process during bone regeneration. More importantly, these studies are expected to validate apoptosis inhibition as a viable therapeutic target for the pharmacological enhancement of bone regeneration. Moreover, the data derived from these studies will add key studies to our existing preliminary data in order to support a competitive R01 focused on the development of new therapeutics designed to enhance normal fracture repair and treat or prevent delayed and non-union fractures.