Temporal and spatial dynamics of sclerostin expression during murine fracture repair

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Introduction: Fracture repair recapitulates embryonic skeletal development, involving both chondro- and osteogenesis. Previous studies have identified the spatial and temporal expression of a myriad of genes altered during fracture repair, including, but not limited to, extracellular matrix genes, transcription and growth factors, and cytokines. It is believed that, by understanding the interdependent stages of healing, novel interventions may be devised to hasten or enhance fracture repair to mechanically competent bone. We examined the expression of sclerostin, an inhibitor of bone formation, during fracture repair. Our results indicate temporal and spatial dynamics of sclerostin expression within the fracture callus.

Materials and Methods: Ten-week-old wild type C57BL/6 mice were used. A closed mid-diaphyseal fracture was made using a three-point bending apparatus with a drop weight. Callus was collected 1, 3, 5, 7, 10, 14, or 21 days post-fracture and processed for histological examination or molecular analysis by quantitative PCR.

Results: Utilizing a standard closed murine fracture model, we examined the spatial and temporal expression of sclerostin, an inhibitor of bone formation. Quantitative PCR analysis revealed low sclerostin expression during the first five days post-fracture (DPF) that began to rise at 7 DPF and reached maximum expression at 10 DPF, after which time expression gradually decreased. Expression of type 10 and type 1 collagen, markers of hypertrophic chondrocytes and osteoblasts, respectively, increased during this time as well, suggesting that both cell types are sources for sclerostin within the fracture callus. Immunofluorescent staining for sclerostin revealed expression in hypertrophic chondrocytes, osteoblasts, and osteocytes at 14 DPF. Osteoblasts and osteocytes continued to express sclerostin at 21 DPF, but there were qualitatively fewer hypertrophic chondrocytes present that expressed sclerostin. These data demonstrate temporal variations in sclerostin expression during fracture repair and, further, indicate differential contributions to total sclerostin expression by chondrogenic and osteogenic cells during fracture repair. Because the fracture site is hypoxic, we hypothesized that alterations in sclerostin expression are mediated by VEGF-A, a potent angiogenic factor whose expression is induced by hypoxia and is vital for normal fracture healing. VEGF-A expression preceded and paralleled changes in sclerostin expression, suggesting that VEGF and hypoxia may provide a level of regulation for sclerostin expression within the fracture callus.

Discussion: Fracture repair is a unique process that restores compromised bone to its original integrity. A thorough understanding of the molecular events involved in fracture repair is vital for the generation of anabolic therapies to combat diseases of bone mass, such as senile osteoporosis. We demonstrate that the expression of sclerostin, a potent inhibitor of bone formation, is spatially and temporally regulated during fracture repair. Sclerostin expression began to rise concomitant with markers of hypertrophic chondrocytes and osteoblasts, suggesting that these cells within the fracture callus are the source of sclerostin. This was confirmed by immunohistochemical staining of sections from 14 and 21 DPF: positive sclerostin immunostaining was observed within hypertrophic chondrocytes, osteoblasts, and osteocytes at 14 DPF, whereas expression was mostly restricted to osteoblasts and osteocytes at 21 DPF. These data indicate differential contribution of chondrogenic and osteogenic cells to the total sclerostin expression observed. Expression of VEGF-A, a potent angiogenic factor, paralleled and preceded changes in sclerostin expression, suggesting that VEGF and hypoxia may provide a level of regulation for sclerostin expression within the fracture callus.