Role of hypoxia inducible factor-1α pathway in bone regeneration

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Keywords: Hypoxia Inducible Factor-1α, von Hippel-Lindau Protein, VEGF, Angiogenesis, Distraction Osteogenesis, Bone Regeneration

Impaired bone repair after trauma, surgery, and/or infections can be a difficult clinical problem with significant cost to the patient and society. Bone regeneration recapitulates processes that occur during skeletal development and requires close temporal and spatial coordination of events involving resident bone cells, marrow stroma and associated vascular elements1-2. Neoangiogenesis is critical for bone regeneration and is dependent of hypoxic stimuli and subsequent pro-angiogenic factors (e.g., VEGF, angiopoietins) production. The hypoxia inducible factor (HIF) pathway is a central pathway for sensing and responding to changes in local oxygen availability in a wide variety of organisms. HIFs impinge on multiple gene programs, which influence angiogenesis, cellular metabolism, cell differentiation and inflammatory response3-4. Consequently, the HIF pathway is ideally suited to coordinate tissue response to injury.

HIF-1α levels are controlled by regulated proteolysis through an oxygen sensitive mechanism. Under normoxic conditions, HIF-1α undergoes prolyl hydroxylation, is ligated by the E3 ubiquitin ligase von Hippel-Lindau protein (pVHL)7-9, and then processed for degradation by the proteasome. The prolyl hydroxylases require oxygen, iron, and 2-oxoglutarate as cofactors10. Under hypoxia, HIF-1α prolyl hydroxylation is inhibited, and HIF-1α then accumulates and translocates into the nucleus where it dimerizes with the HIF-1β subunit (also known as aryl hydrocarbon receptor nuclear translocator). The dimer then complexes with coactivator p300 and transactivates genes having proximal promoter regions containing hypoxic response elements (HREs)11.

Our group has recently shown that HIFs are required for normal skeletal development12. To investigate the role of HIF-1α during bone regeneration, we have performed distraction osteogenesis (DO) in mutant mice with constitutive activation or inactivation of HIF-1α in bone as a result of deletion of VHL or HIF-1α in osteoblasts, respectively. In addition, we examined whether small molecules that inhibit the prolyl hydroxylases (PHDs) involved in degrading HIFs could activate the HIF pathway in vitro, and stimulate angiogenesis and improve bone healing in vivo. The data showed that the HIF-1 pathway was activated during bone repair and can be manipulated genetically and pharmacologically to improve skeletal healing13. Mice lacking pVHL in osteoblasts with constitutive HIF-1α activation in osteoblasts had markedly increased vascularity and produced more bone in response to DO whereas mice lacking HIF-1α in osteoblasts had impaired angiogenesis and bone healing. The increased vascularity and bone regeneration in the pVHL mutants were VEGF-dependent and eliminated by concomitant administration of VEGF receptor antibodies. Small molecule inhibitors of HIF prolyl hydroxylation stabilized HIF/VEGF production and increased angiogenesis in vitro. One of these molecules (DFO) administered in vivo into the distraction gap increased angiogenesis and markedly improved bone regeneration. These results identify the HIF-1α pathway as a critical mediator of neoangiogenesis required for skeletal repair and suggest the application of HIF activators as therapies to improve bone regeneration.

Future studies are underway to examine the cell signaling pathways that control mesenchymal stem cell fate determination under hypoxic microenvironment of bone regenerate, and the mechanisms that regulate the coupling of angiogenesis and osteogenesis during bone regeneration. Our long-term goals focus on exploring novel therapeutic approaches and agents to facilitate bone repair.
References


