

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

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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 elements^{1,2}. 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 response³⁻⁶. Consequently, the HIF pathway is ideally suited to coordinate tissue response to injury.

HIF-1 α levels are controlled by regulated proteolysis through an oxygen sensitive mechanism. Under normoxic conditions, HIF-1 α 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-1 α prolyl hydroxylation is inhibited, and HIF-1 α then accumulates and translocates into the nucleus where it dimerizes with the HIF-1 β 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-1 α during bone regeneration, we have performed distraction osteogenesis (DO) in mutant mice with constitutive activation or inactivation of HIF-1 α in bone as a result of deletion of VHL or HIF-1 α 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-1 α activation in osteoblasts had markedly increased vascularity and produced more bone in response to DO whereas mice lacking HIF-1 α 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-1 α 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.

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References

1. Ferguson C, Alpern E, Miclau T, Helms JA. Does adult fracture repair recapitulate embryonic skeletal formation? *Mech Dev* 1999;87:57-66.
2. Gerstenfeld LC, Cullinane DM, Barnes GL, Graves DT, Einhorn TA. Fracture healing as a post-natal developmental process: molecular, spatial, and temporal aspects of its regulation. *J Cell Biochem* 2003; 88:873-84.
3. Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, Capla JM, Galiano RD, Levine JP, Gurtner GC. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med* 2004;10:858-64.
4. Cramer T, Yamanishi Y, Clausen BE, Forster I, Pawlinski R, Mackman N, Haase VH, Jaenisch R, Corr M, Nizet V, Firestein GS, Gerber HP, Ferrara N, Johnson RS. HIF-1 α is essential for myeloid cell-mediated inflammation. *Cell* 2003;112:645-57.
5. Robins JC, Akeno N, Mukherjee A, Dalal RR, Aronow BJ, Koopman P, Clemens TL. Hypoxia induces chondrocyte-specific gene expression in mesenchymal cells in association with transcriptional activation of Sox9. *Bone* 2005;37:313-22.
6. Yun Z, Maecker HL, Johnson RS, Giaccia AJ. Inhibition of PPAR γ 2 gene expression by the HIF-1-regulated gene DEC1/Stra13: a mechanism for regulation of adipogenesis by hypoxia. *Dev Cell* 2002; 2:331-41.
7. Kallio PJ, Wilson WJ, O'Brien S, Makino Y, Poellinger L. Regulation of the hypoxia-inducible transcription factor 1 α by the ubiquitin-proteasome pathway. *J Biol Chem* 1999;274:6519-25.
8. Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara JM, Lane WS, Kaelin WG Jr. HIF1 α targeted for VHL-mediated destruction by proline hydroxylation: implications for O₂ sensing. *Science* 2001; 292:464-8.
9. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER, Ratcliffe PJ. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* 1999;399:271-5.
10. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, Kriegsheim A, Hestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW, Ratcliffe PJ. Targeting of HIF- α to the von Hippel-Lindau ubiquitylation complex by O₂-regulated prolyl hydroxylation. *Science* 2001;292:468-72.
11. Kallio PJ, Okamoto K, O'Brien S, Carrero P, Makino Y, Tanaka H, Poellinger L. Signal transduction in hypoxic cells: inducible nuclear translocation and recruitment of the CBP/p300 coactivator by the hypoxia-inducible factor-1 α . *EMBO J* 1998;17:6573-86.
12. Wang Y, Wan C, Deng L, Liu X, Cao X, Gilbert SR, Bouxsein ML, Faugere MC, Guldberg RE, Gerstenfeld LC, Haase Vh, Johnson Rs, Schipani E, Clemens TL. The hypoxia-inducible factor α pathway couples angiogenesis to osteogenesis during skeletal development. *J Clin Invest* 2007;117:1616-26.
13. Wan C, Gilbert SR, Wang Y, Cao XM, Shen X, Ramaswamy G, Jacobsen KA, Alaql ZS, Gerstenfeld LC, Einhorn TA, Guldberg RE, Deng L, Clemens TL. Activation of the hypoxia-inducible factor-1 α pathway accelerates bone regeneration. *Proc Natl Acad Sci USA*. 2008;105:686-91.