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Posttranslational modifications of collagens as targets of hypoxia and Hif-1α in endochondral bone development

Ernestina Schipani
Endocrine Unit, MGH-Harvard Medical School, Boston, Massachusetts, USA

Abstract

In recent years, it has been proposed that oxygen is not only an indispensable metabolic substrate, but also a regulatory signal, and that gradients of oxygenation turn on a specific genetic program. A crucial mediator of the adaptive response of cells to hypoxia is the transcription factor hypoxia-inducible factor 1*α* (Hif-1α). The fetal growth plate, which is an avascular structure of mesenchymal origin, has a unique out-in gradient of oxygenation. Hif-1α is required for chondrogenesis *in vivo* by controlling a complex homeostatic response that allows chondrocytes to survive and differentiate in an hypoxic environment. Preliminary evidence suggests that regulation of posttranslational modifications of collagens could be an important component of this adaptive response.

Keywords

hypoxia; Hif-1α; VHL; chondrocytes; matrix; collagen

Introduction

Hypoxia is a relative decrease of oxygen (O_2) availability. The definition of "physiologically" normoxic conditions for either embryonic or adult cells varies significantly. Before the circulatory system is established, mammalian development proceeds in a relatively low O_2 environment of about 3%.[1] Moreover, studies that have utilized small-molecule hypoxia markers have shown the existence of specific regions of moderate to severe hypoxia in the developing embryos.[2] In the majority of normal adult tissues, oxygen (O_2) levels vary between 2% and 9% (as compared to ambient air, which contains 21% O_2).[3] In contrast, O_2 concentrations in regions of the bone marrow, cartilage, kidney medulla, and thymus are below 1% O_2.[3] Hypoxia is not only a critical factor in fetal development and differentiation, but also a pathophysiologic component of many human disorders, including cancer and ischemic diseases.[4]

Hypoxia-inducible factor-1 (Hif-1), a ubiquitously expressed transcription factor, is a major regulator of cellular adaptation to hypoxia.[5–8] It is a heterodimeric DNA-binding complex that consists of two basic helix-loop-helix (bHLH) proteins of the PER/ARNT/SIM (PAS) subfamily, Hif-1α and Hif-1β. Hif-1α and Hif-1β mRNAs are ubiquitously expressed. Hif-1α levels increase exponentially as O_2 levels drop below 5%. On the other hand, Hif-1β (also known as aryl hydrocarbon nuclear translocator or ARNT) is non-oxygen-responsive.

Address for correspondence: Ernestina Schipani, M.D., Ph.D., Massachusetts General Hospital, Endocrine Unit, Thier Research Building, Room 1101, 50 Blossom Street, Boston, MA 02114. schipani@helix.mgh.harvard.edu.

Conflicts of interest
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Upon heterodimerization with Hif-1β, the Hif-1α/Hif-1β complex binds to a specific sequence 5′-RCGTG-3′ (where R denotes a purine residue) termed *hypoxia response elements* (HREs), and transactivates target genes containing HREs. Hif-1α does not directly sense variations of O_2 tension; a class of 2-oxoglutarate-dependent and Fe^{2+}-dependent dioxygenases are the O_2 sensors.[9] Two types of O_2 sensors are involved in Hif-1α action, prolyl-hydroxylase domain proteins (PHDs) and an asparaginyl hydroxylase, respectively. PHDs hydroxylate two prolyl residues (P402 and P564) in the HIF-1α region, referred to as the O_2-dependent degradation domain (ODDD). This modification occurs in normoxic conditions and mediates the binding of the von Hippel-Lindau tumor suppressor protein (pVHL), which is an E3 ubiquitin ligase, to Hif-1α. Hif-1α s then marked with polyubiquitin chains and targeted for degradation by the proteasome. In well-oxygenated tissues, where O_2 tension is higher than 5%, Hif-1α displays one of the shortest half-lives (< 5 min) among cellular proteins. Conversely, under hypoxic conditions, the activity of the PHDs is largely impaired and proline hydroxylation cannot occur. As a result, Hif-1α protein accumulates and this initiates a multi-step pathway that includes nuclear translocation of Hif-1α, dimerization with its partner Hif-1β, recruitment of transcriptional co-activators, and binding to HREs within the promoters of hypoxia-responsive genes. The second type of O_2 sensor is an asparaginyl hydroxylase called factor-inhibiting Hif-1 (FIH-1). This enzyme hydroxylates an asparagine residue (N803) in the carboxy-terminal transcriptional activation domain (C-TAD) of Hif-1α. This covalent modification blocks C-TAD interaction with transcriptional co-activators, such as p300 and CBP. Thus, the two O_2 sensors, PHD and FIH, by regulating the destruction and activity of Hif-1α, respectively, ensure the repression of the Hif-1 pathway in well-oxygenated cells.

To date, more than 100 putative Hif-1 target genes have been identified.[10–12] They are involved in a wide variety of biological processes including energy metabolism, angiogenesis, erythropoiesis, cell survival, apoptosis, redox, and pH regulation.[11] Mouse embryos lacking Hif-1α exhibit multiple morphologic defects as early as embryonic day E8.5, and die *in utero* by E10.5.[13–15] Many malignant cancers contain regions of severe hypoxia, resulting in high levels of Hif-1α that drive tumor progression,[5,8] and inhibition of Hif-1α has been proposed as a potentially powerful approach.

Hif-1α is necessary for chondrocyte survival and differentiation and regulates joint development

The fetal growth plate is a striking example of the critical and nonredundant role of the transcription factor Hif-1α in survival and differentiation of hypoxic cells *in vivo*.

Skeletal development depends on two mechanisms, intramembranous and endochondral.[16] In the first, mesenchymal cells develop directly into osteoblasts and form the flat bones of the skull. The second, accounting for the development of most other bones, involves a two-stage mechanism, whereby chondrocytes form a matrix template, the growth plate, which is then replaced by bone. During endochondral bone development, growth plate chondrocytes undergo well-ordered and controlled phases of cell proliferation, maturation, and death. Proliferative chondrocytes synthesize collagen type II and form a columnar layer. They then stop proliferating and differentiate into postmitotic hypertrophic cells. Hypertrophic chondrocytes express predominantly collagen type X and mineralize their surrounding matrix. Differentiation is followed by death of hypertrophic chondrocytes, followed by blood vessel invasion, and finally by replacement of the cartilaginous matrix with bone.

The cartilaginous matrix is formed by two components, the proteoglycans and the collagens. Proteoglycans are macromolecules containing a core protein with multiple attached polysaccharide chains.[17] Because of their high content of charged polysaccharides,

proteoglycans are highly hydrated. In regard to collagens, there are three different types of collagens in the growth plate matrix, fibrillar, fibril-associated, and sheet-forming collagens.[18] Fibrillar collagens comprise collagen type II and collagen type XI. Collagen type II is also found in the vitreous. In cartilage, collagen type II is produced by proliferating chondrocytes and by the upper hypertrophic chondrocytes. Fibril-associated collagens comprise collagen type IX, which, as collagen type II, is also found in the vitreous and binds proteoglycans. Sheet-forming collagens comprise collagen type X. Collagen type X is exclusively expressed by hypertrophic chondrocytes.

Cartilage is an avascular and hypoxic mesenchymal tissue,[19] although it requires an angiogenic switch in order to be replaced by bone. The fetal growth plate contains a hypoxic central region.[19] By using the marker of hypoxia EF5, the fetal chondrocytic growth plate has been shown to bind EF5, with no binding detected in surrounding soft tissues. The most hypoxic chondrocytes are in the round proliferative layer near the joint space, in the center of the columnar proliferative layer and in the upper portion of the hypertrophic zone.[19] The EF5 findings document a gradient of oxygenation, from the proliferative to the hypertrophic zone, as well as from the outer to the inner region of the fetal growth plate. The high rate of O_2 diffusion to the mineralized hypertrophic layer may be the reason for the hypoxic condition of the early hypertrophic chondrocytes, even though they are located near the blood vessels of the primary spongiosa.[19]

Analysis of *in vivo* genetic models using the Cre-LoxP system has shown that Hif-1α is necessary for chondrogenesis *in vivo* by turning on a complex homeostatic response that allows chondrocytes to survive and differentiate in a hypoxic environment.[19,20]

Moreover, Hif-1α protein is also particularly abundant in the highly hypoxic developing joints, possibly because the avascular perichondrium surrounding them is thickened.[20] Even after the joint space has formed, articular chondrocytes are significantly more hypoxic than the rest of the cartilage.[20] Lack of Hif-1α in limb bud mesenchyme delays joint development, without altering thickening of the perichondrium.[20,21] Thickening of the perichondrium therefore precedes joint formation and is likely to be critical for joint development. The delay of early chondrogenesis, secondary to the lack of Hif-1α, may impair joint formation. However, because of the pronounced expression of Hif-1α in the prospective joint, the delayed joint formation associated with loss of Hif-1α may not be the only consequence of a delay in early chondrogenesis.

The role of Hif-1α in cell differentiation is tissue-specific, because Hif-1α maintains stem cells in an undifferentiated state[3,22–25] and inhibits differentiation of mesenchymal cells into osteoblasts, adipocytes, and myocytes,[3,26–28] yet stimulates differentiation of trophoblastic cells and dopaminergic neurons and chondrocytes.[3,29–31]

Role of posttranslational modifications of collagens in mediating the survival and differentiation functions of Hif-1α in growth plate chondrocytes

A variety of mechanisms can be invoked downstream of Hif-1α as a survival and differentiation factor, including regulation of VEGF expression and modulation of metabolic pathways and autophagy.[20,32] However, recent experimental evidence indicates that regulation of posttranslational modification of collagens, with hydroxylation of collagen prolines in particular, could be one of the modalities by which Hif-1α regulates chondrocyte survival and differentiation. Prolyl-4-hydroxylases I and II (P4HaI and P4HaII) are the enzymes responsible for generating 4-hydroxyprolines in the collagens; these are essential for the formation of triple-helical collagens. P4Has have much lower K_m for O_2 than the

PHDs, which trigger Hif-1α degradation (20 versus 250, respectively).[33] This indicates that P4Has require a minimal amount of O_2 for proper function, that is, they still function enzymatically at low O_2 levels. The alpha subunits of P4HaI and P4HaII are targets of hypoxia in chondrocytes and other cell types in a Hif-1α-dependent fashion.[20,34] Proper accumulation of extracellular matrix is not only essential for organ development, but also promotes cell differentiation and survival through specific cell-matrix interactions.[35] Hif-1α may thus operate as a survival and differentiation factor in chondrocytes, improving the efficiency of posttranslational modifications of collagen type II and, in so doing, promoting the formation of a proper extracellular matrix. A defect in posttranslational hydroxylation of collagens leads to a decrease in extracellular matrix and an increase in under-hydroxylated collagens. This in turn may trigger an unfolded protein response (UPR),[36] and may be a cause of the delayed chondrogenesis in mice that lack Hif-1α in limb bud mesenchyme.

The positive effect of Hif-1α on matrix accumulation in chondrocytes is consistent with the role of hypoxia in promoting fibrosis in pathologic conditions.[37] Moreover, it has been reported that lysyl oxidase, which is responsible for the formation of cross-links between collagen molecules, is induced by hypoxia and is essential for metastasis of highly malignant and hypoxic tumors.[38]

Hypoxia and Hif-1α may also modulate matrix formation by chondrocytes by upregulating expression of Sox9,[21,39] a master regulator of chondro-genesis.[40] In mouse bone marrow stromal (ST2) cells, in particular, hypoxia brings about an increase in nuclear accumulation of Hif-1α and Sox9 transcription.[39] Similar findings have been reported in limb bud micromass cultures,[21] but not in primary chondrocytes or *ex vivo* metatarsal explants.[20] Notably, no evidence has been reported of a role of hypoxia in directly regulating expression of mRNAs encoding collagens in growth plate chondrocytes.[20]

Summary

In this brief review, we have highlighted the role of hypoxia and Hif-1α in regulating posttranslational modifications of collagens, and its importance in mediating the survival and differentiation functions of HIF-1α in the developing cartilage and joints. It will be now important to investigate whether regulation of collagen P4Hs is the main mechanism adopted by this transcription factor to control matrix accumulation in the developing growth plate. To study regulation of posttranslational modifications of collagens by hypoxia and Hif-1α in cartilage and joints may significantly expand our understanding of cellular adaptation to hypoxia as well as cartilage homeostasis.

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References

1. Rodesch F, et al. Oxygen measurements in endometrial and trophoblastic tissues during early pregnancy. Obstet Gynecol 1992;80:283–285. [PubMed: 1635745]
2. Chen EY, Fujinaga M, Giaccia AJ. Hypoxic microenvironment within an embryo induces apoptosis and is essential for proper morphological development. Teratology 1999;60:215–225. [PubMed: 10508975]
3. Simon MC, Keith B. The role of oxygen availability in embryonic development and stem cell function. Nat Rev Mol Cell Biol 2008;9:285–296. [PubMed: 18285802]
4. Giaccia AJ, Simon MC, Johnson R. The biology of hypoxia: the role of oxygen sensing in development, normal function, and disease. Genes Dev 2004;18:2183–2194. [PubMed: 15371333]

5. Giaccia A, Siim B, Johnson R. HIF-1 as a target for drug development. Nat Rev Drug Discov 2003;2:803–811. [PubMed: 14526383]
6. Kaelin W. How oxygen makes its presence felt. Genes Dev 2002;16:1441–1445. [PubMed: 12080083]
7. Liu L, Simon MC. Regulation of transcription and translation by hypoxia. Cancer Biol Ther 2004;3:492–497. [PubMed: 15254394]
8. Semenza GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer 2003;3:721–732. [PubMed: 13130303]
9. Pouyssegur J, Dayan F, Mazure NM. Hypoxia signalling in cancer and approaches to enforce tumour regression. Nature 2006;441:437–443. [PubMed: 16724055]
10. Leo C, Giaccia A, Denko N. The hypoxic tumor microenvironment and gene expression. Semin Radiat Oncol 2004;14:207–214. [PubMed: 15254863]
11. Greijer AE, et al. Up-regulation of gene expression by hypoxia is mediated predominantly by hypoxia-inducible factor 1 (HIF-1). J Pathol 2005;206:291–304. [PubMed: 15906272]
12. Bishop T, et al. Genetic analysis of pathways regulated by the von Hippel-Lindau tumor suppressor in *Caenorhabditis elegans*. PLoS Biol 2004;2:e289. [PubMed: 15361934]
13. Ryan HE, Lo J, Johnson RS. HIF 1 alpha is required for solid tumor formation and embryonic vascularization. EMBO J 1998;17:3005–3015. [PubMed: 9606183]
14. Iyer N, et al. Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1alpha. Genes Dev 1998;12:149–162. [PubMed: 9436976]
15. Compernolle V, et al. Cardia bifida, defective heart development and abnormal neural crest migration in embryos lacking hypoxia-inducible factor -1alpha. Cardiovascular Res 2003;60:569–79.
16. Karsenty G. The complexities of skeletal biology. Nature 2003;423:316–318. [PubMed: 12748648]
17. Schwartz N, Domowicz M. Chondrodysplasias due to proteoglycan defects. Glycobiology 2002;12:57R–68R.
18. Olsen BR. Role of cartilage collagens in formation of the skeleton. Ann NY Acad Sci 1996;785:124–130. [PubMed: 8702118]
19. Schipani E, et al. Hypoxia in cartilage: HIF-1alpha is essential for chondrocyte growth arrest and survival. Genes Dev 2001;15:2865–2876. [PubMed: 11691837]
20. Provot S, et al. Hif-1alpha regulates differentiation of limb bud mesenchyme and joint development. J Cell Biol 2007;177:451–464. [PubMed: 17470636]
21. Amarilio R, et al. Hif1alpha regulation of Sox9 is necessary to maintain differentiation of hypoxic pre-chondrogenic cells during early chondrogenesis. Development 2007;134:3917–3928. [PubMed: 17913788]
22. Jeong C, et al. Hypoxia-inducible factor -1alpha inhibits self-renewal of mouse embryonic stem cells in vitro via negative regulation of the leukemia inhibitory factor-STAT3 pathway. J Biol Chem 2007;282:13672–13679. [PubMed: 17360716]
23. Lin Q, Lee Y, Yun Z. Differentiation arrest by hypoxia. J Biol Chem 2006;281:30678–30683. [PubMed: 16926163]
24. Sainson R, Harris A. Hypoxia-regulated differentiation: let's step it up a Notch. Trends Mol Med 2006;12:141–143. [PubMed: 16513423]
25. Gustafsson M, et al. Hypoxia requires Notch signaling to maintain the undifferentiated cell state. Dev Cell 2005;9:617–628. [PubMed: 16256737]
26. Salim A, et al. Transient changes in oxygen tension inhibit osteogenic differentiation and Runx2 expression in osteoblasts. J Biol Chem 2004;279:40007–40016. [PubMed: 15263007]
27. Yun Z, Lin Q, Giaccia A. Adaptive myogenesis under hypoxia. Mol Cell Biol 2005;25:3040–3055. [PubMed: 15798192]
28. Yun Z, et al. Inhibition of PPAR gamma 2 gene expression by the HIF-1-regulated gene DEC-1/Stra13 a mechanism for regulation of adipogenesis by hypoxia. Dev Cell 2002;2:331–341. [PubMed: 11879638]
29. Dahl KC, et al. Hypoxia-inducible factors 1alpha and 2alpha regulate trophoblast differentiation. Mol Cell Biol 2005;25:10479–10491. [PubMed: 16287860]

30. Morrison SJ, et al. Culture in reduced levels of oxygen promotes clonogenic sympathoadrenal differentiation by isolated neural crest stem cells. J Neurosci 2000;20:7370–7376. [PubMed: 11007895]

31. Studer L, et al. Enhanced proliferation, survival, and dopaminergic differentiation of CNS precursors in lowered oxygen. J Neurosci 2000;20:7377–7383. [PubMed: 11007896]

32. Khatri R, Schipani E. About the importance of being desulfated. Genes Dev 2008;22:2750–2754. [PubMed: 18923073]

33. Hirsila M, et al. Characterization of the human prolyl 4-hydroxylases that modify the hypoxia-inducible factor. J Biol Chem 2003;278:30772–30780. [PubMed: 12788921]

34. Grimmer C, et al. Regulation of type II collagen synthesis during osteoarthritis by prolyl-4-hydroxylases: possible influence of low oxygen tension. Am J Pathol 2006;169:491–502. [PubMed: 16877351]

35. Egerbacher M, Haeusler G. Integrins in growth plate cartilage. Pediatr Endocrinol Rev 2003;1:2–8. [PubMed: 16437008]

36. Tsang K, et al. Surviving endoplasmic reticulum stress is coupled to altered chondrocyte differentiation and function. PLoS Biol 2007;5:e44. [PubMed: 17298185]

37. Higgins DF, et al. Hypoxia-inducible factor signaling in the development of tissue fibrosis. Cell Cycle 2008;7:1128–1132. [PubMed: 18418042]

38. Erler JT, et al. Hypoxia-induced lysyl oxidase is a critical mediator of bone marrow cell recruitment to form the premetastatic niche. Cancer Cell 2009;15:35–44. [PubMed: 19111879]

39. Robins JC, et al. Hypoxia induces chondrocyte-specific gene expression in mesenchymal cells in association with transcriptional activation of Sox9. Bone 2005;37:313–322. [PubMed: 16023419]

40. Lefebvre V, Smits P. Transcriptional control of chondrocyte fate and differentiation. Birth Defects Res C Embryo Today 2005;75:200–212. [PubMed: 16187326]