HIF-2α as a possible therapeutic target of osteoarthritis

T. Saito ‡‡, H. Kawaguchi ‡‡†

‡‡Bone & Cartilage Regenerative Medicine, Faculty of Medicine, University of Tokyo, Tokyo 113-8655, Japan
‡‡Sensory & Motor System Medicine, Faculty of Medicine, University of Tokyo, Tokyo 113-8655, Japan

SUMMARY

Objective: Endochondral ossification, a conversion process from nonvascularized and hypoxic cartilage to highly vascularized bone, plays a crucial role in osteoarthritis (OA) development as well as in physiological skeletal growth. We have shown that hypoxia-inducible factor-2α (HIF-2α, encoded by EPAS1) is an extensive regulator of the endochondral ossification process. Here we review the possible signaling network regulating OA development on the axis of HIF-2α.

Methods: Peer reviewed publications published prior to August 2010 were searched in the Pubmed database. Articles that were relevant to HIF and molecular mechanisms of the endochondral ossification and OA were selected.

Results: As a trigger of OA, mechanical stress may induce the upstream NF-κB signal and HIF-2α expression in joint cartilage of mice and humans, which causes transactivation of endochondral ossification-related molecules with the most potent β-subunit partner aryl hydrocarbon nuclear translocator-like (ARNTL). In contrast to HIF-2α, HIF-1α functions to maintain cartilage via a distinct mechanism, so that the shifting of the HIFs might possibly be involved in an OA pathogenesis.

Conclusion: Signals on the HIF-2α axis from NF-κB signaling to the endochondral ossification-related molecules, possibly in combination with HIF-2α and ARNTL, may represent a rational therapeutic target for OA with minimal effects on physiological skeletal homeostasis.

© 2010 Osteoarthritis Research Society International. Published by Elsevier Ltd. All rights reserved.
(HIF-2α, encoded by EPAS1), an α-subunit member of the HIF family, as the most potent transactivator41. Here we review the possible signaling network regulating OA development on the axis of HIF-2α.

**Physiological roles of HIF proteins in cartilage**

The HIF proteins belong to the basic-helix-loop-helix/Per-ARNT-Sim (bHLH/PAS) transcription factor family, and consist of α- and β-subunit members22,23. Under normoxic conditions, the α-subunit members HIF-1α, 2α and 3α undergo oxygen-dependent hydroxylation, resulting in ubiquitination and degradation by the proteasome4,42,43. In contrast, under hypoxic conditions, they are neither hydroxylated nor degraded, and heterodimerize with the constitutive β-subunit members. The heterodimers activate transcription of the target genes by binding the consensus sequence called hypoxia-responsive element in the promoters45. In fact, HIF-1α plays essential roles to assure survival under hypoxic conditions in various tissues by regulating vascularization, endothelial cell proliferation, iron metabolism, and glucose metabolism22. Although all HIF-2α is approximately 50% homologous to HIF-1α at the amino acid level, their distributions, target genes, and functions are notably different26–28. HIF-1α is expressed mainly in hypoxic, poorly vascularized sites, while HIF-2α is also expressed in well-vascularized tissues7,28.

In cartilage as well, the hypoxic condition enhances the expression and activity of HIF-1α24,29–31. HIF-1α is extensively localized in chondrocytes from the earlier differentiation stage21,29–31, and induces initial chondrogenesis, joint development, cartilage matrix synthesis, and cell survival29–33. In contrast, HIF-2α is expressed mainly in highly differentiated chondrocytes, and its function is independent of oxygen-dependent hydroxylation, denying the importance of HIF-2α for survival of hypoxic conditions in cartilage21,34,35. Likewise, although cartilage-specific knockout of HIF-1α leads to defects in cartilage formation and chondrocyte survival31,33, HIF-2α-haploinsufficient mice exhibit mild and transient dwarfishness at the embryonic stage with an elongation of the hypertrophic zone and delayed ossification in the limb cartilage, indicating that HIF-2α insufficiency impairs not only chondrocyte hypertrophy but also subsequent extensive steps of endochondral ossification. In fact, HIF-2α induces expressions of many key factors for these steps: COL10A1, MMP-13, MMP-3, MMP-9, runt-related transcription factors like RUNX2, IHH and VEGF21. RUNX2 is a mediator of osteogenic differentiation in highly differentiated chondrocytes, just like HIF-2α21. Although all β-subunit members are physically associated with HIF-2α as previously reported20–43, ARNTL shows the strongest binding affinity to HIF-2α and enhancement of the HIF-2α transactivation of COL10A1, MMP13 and VEGFA21. Meanwhile, ARNTL-null mice show impaired circadian rhythms and ectopic ossification with age without abnormality in skeletal development or joint cartilage44. Hence, ARNTL seems to be the most potent β-subunit partner of HIF-2α in chondrocytes, although all β-subunit members may be involved in the regulation of HIF-2α function in cartilage.

**Roles of HIF-2α in OA development**

Chun’s group and ours have recently described a central role of HIF-2α in the OA development21,35. HIF-2α is highly expressed in the OA cartilage of the mouse experimental model and human surgical specimens. Despite the mild and transient phenotype in the embryonic skeletal development under physiological conditions, HIF-2α-haploinsufficient mice show a marked resistance to cartilage degradation and osteophyte formation under the OA induction in the knee joints with decreased expressions of endochondral ossification-related factors as well as catabolic factors including MMPs, nitric oxide synthase-2 (NOS2) and prostaglandin-endoperoxide synthase-2 (PTGS2)21,35,45. In contrast, cartilage destruction is enhanced in mice with chondrocyte-specific overexpression of HIF-2α and with intra-articular injection of the HIF-2α adenovirus, accompanied by increased expressions of the catabolic factors21. Since the mouse OA model mimics the clinical OA, using a Japanese population-based cohort of the ROAD study45, we have identified a functional single nucleotide polymorphism (SNP) in the human HIF-2α gene which is associated with knee OA21.

OA reflects an imbalance between matrix anabolic and catabolic processes in joint cartilage, so that the protection is achieved by induction of anabolism or inhibition of catabolism. The protection in the HIF-2α-haploinsufficient mice is not likely to be due to induction of anabolism, as the anabolic markers like type II collagen and aggrecan are unaffected in the joint cartilage21,35. Because MMP-13-null mice are reported to be protected from cartilage degradation after surgical OA induction4, similarly to HIF-2α-haploinsufficient mice, the OA protection caused by the HIF-2α insufficiency might occur principally through regulation of MMP-13. Notably, although recent studies have identified ADAMTS-5 and related molecules like syndecan-4 as key catabolic regulators of OA development4,5,46,47, ADAMTS-5 is not altered by the overexpression or knockout of HIF-2α, implicating an independent pathway21,35. Besides catabolic factors, HIF-2α induces osteogenic factors like RUNX2, IHH and VEGF21. RUNX2 is a mediator of chondrocyte hypertrophy and of MMP-13 expression just weeks into the course of the experimental mouse knee OA model14. However, RUNX2 does not seem to be essential for the OA induction by HIF-2α, since overexpression of the dominant negative mutant does not affect the HIF-2α functions47. The IHH signal is also shown to promote OA development as well as the physiological skeletal growth, although being partly mediated by RUNX2 expression47. VEGF is a well-characterized growth factor inducing angiogenesis, and a representative target gene of HIF-1α31,48. VEGF may be a major mediator for the osteophyte formation induced by HIF-2α, since this is highly expressed in the periphery of OA cartilage21. Chun’s group and ours agree that the NF-κB signal is an upstream mechanism that regulates HIF-2α, since IL-1β and TNF-α, putative ligands for the NF-κB signal, increase the HIF-2α expression in chondrocytes21,35.

A screening using the HIF-2α promoter assay identified v-rel reticuloendotheliosis viral oncogene homolog A (RelA or NF-κB p65), an NF-κB family member, as the most potent transactivator, and determined an NF-κB motif as the core responsive region by mutagenesis analysis21. The RelA expression was induced alongside the HIF-2α expression during OA development, and the SNP described above is involved in the HIF-2α induction by the NF-κB signal. Considering that the NF-κB signal is activated not only by inflammatory stimulation, but also by...
mechanical stress\textsuperscript{49,50}, the NF-κB / HIF-2α signal may be a major mediator from mechanical stress to OA development in joint cartilage.

Since HIF-1α functions to maintain cartilage while HIF-2α to induce endochondral ossification and cartilage degradation, the shift from HIF-1α to HIF-2α might possibly be a pathogenesis of OA\textsuperscript{29–31,51,52} (Fig. 1). A recent study shows that the autophagic response is promoted by HIF-1α and antagonized by HIF-2α in chondrocytes\textsuperscript{53}. Due to the difference of sensitivity to the oxygen level between HIF-1α and HIF-2α, the oxic conditions in cartilage may regulate the shift of the HIF proteins. Hence, prolyl hydroxylases which become active in the presence of oxygen and target the HIFs for hydroxylation and degradation may be involved in the OA pathogenesis.

Prospects

HIF-2α is an extensive regulator of endochondral ossification process during OA development\textsuperscript{21}. As a trigger of OA, mechanical stress may induce the upstream NF-κB signal and HIF-2α expression in joint cartilage, which cause endochondral ossification by trans-activation of COL10A1, MMP13, VEGFA and other osteogenic factors. Signals in the HIF-2α axis from NF-κB signaling to the endochondral ossification-related molecules may represent a rational therapeutic target for OA with minimal effects on physiological skeletal homeostasis. However, there are several issues to be resolved before the clinical application to human OA treatment. First, HIF-2α in cartilage may be difficult to target with a systemic drug because cartilage lacks vasculature. Moreover, since HIF-2α is known to have physiological protective functions against neuronal oxidative stress and normoxic oxidative neuronal death\textsuperscript{54}, the safety margin for systemic administration of a HIF-2α inhibitor may be limited. One solution to these challenges might be to administer the HIF-2α inhibitor by intra-articular injection. Although joint cartilage cells would take up the injected inhibitor via diffusion from synovial fluid and surrounding joint tissue, transcription factors are still difficult to target. Hence, we need to establish a suitable drug delivery system which is effective and selective to chondrocytes. Secondly, although HIF-2α expression is induced in the earlier stage of OA lesions, it is downregulated in the late stages\textsuperscript{21,35}, suggesting that the time window for using the inhibitor may be narrow. For the late stage of OA, anabolic signals related to HIF-1α might be useful. Modulation of ARNTL which functions as a potent partner of HIF-2α but not of HIF-1α\textsuperscript{21} might be useful for the switching from HIF-2α to HIF-1α. Further studies of chondrocyte differentiation and potential regulatory effects of chondrocyte microRNAs\textsuperscript{55,56} on the HIF protein expressions may also merit further investigation. Lastly, it may be more efficient to identify the extracellular signal that either directly activates or suppresses HIF-2α, because this might be easier to target than intracellular signals.

Author contributions

Taku Saito and Hiroshi Kawaguchi: conception, drafting, critical revision and final approval of the manuscript.

Conflicts of interest

None.

Acknowledgements

This study was supported by a grant-in-aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology (19109007 and 20689028). The sponsor had no role in study design, data collection, data analysis, data interpretation or writing of the manuscript.

References


