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Review

HIF-2α as a possible therapeutic target of osteoarthritis

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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 endochondal 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.
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Introduction

Although osteoarthritis (OA) is today considered a major public health issue causing chronic disability worldwide in the increasing number of aging people[1,2], this disorder is far behind other skeletal diseases like osteoporosis and rheumatoid arthritis in the disease-modifying treatments which have been developed. This is mainly because little is known about the underlying molecular mechanism which is the therapeutic target. Recent studies using experimental mouse models with surgical instability in the knee joints have shown that OA is initiated by production of proteases like matrix metalloproteinase-13 (MMP-13) and a disintegrin and metalloproteinase with thrombospondin motif-5 (ADAMTS-5)[3–5]. However, trials applying the protease inhibitors for clinical use as a disease-modifying treatment have to date been unsuccessful because of insufficient efficiency and adverse events[6–8]. Hence, a number of alternative or complementary approaches to selective protease inhibition merit consideration.

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Endochondral ossification process in OA development

We and others have reported that the endochondral ossification process plays a crucial role not only in physiological skeletal growth, but also in OA development[9–19]. In other words, OA involves a reinitiation of the process that normally occurs in embryos and children and ceases in adulthood. This process starts with hypertrophic differentiation of chondrocytes characterized by type X collagen (COL10A1) expression, followed by the conversion of nonvascularized and hypoxic cartilage tissue into highly vascularized bone tissue *via* degradation of the cartilage matrix and vascular invasion[20]. The signals to induce endochondral ossification cause cartilage degradation at the center of the joint and osteophyte formation at the periphery. The difference of the two sites may depend on the vascularity. At the periphery, vascularity is accessible from the synovium or tendon, which completes endochondral ossification and forms osteophytes, just as it does at the embryonic and growth plate cartilage. However, in the center, the vascularity is not accessible from the edge, remaining cartilage degradation without being replaced by bone[15,16].

Aiming at identification of therapeutic targets for OA, we have performed a screen of transcription factors that potentiate the expression of *COL10A1* and identified hypoxia-inducible factor-2α

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(HIF-2α, encoded by *EPAS1*), an α-subunit member of the HIF family, as the most potent transactivator[21]. 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 members[22,23]. Under normoxic conditions, the α-subunit members HIF-1α, 2α and 3α undergo oxygen-dependent hydroxylation, resulting in ubiquitination and degradation by the proteasome[24,25]. 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 promoters[23]. In fact, HIF-1α plays essential roles to assure survival under hypoxic conditions in various tissues by regulating vascularization, erythropoiesis iron metabolism, and glucose metabolism[22]. Although HIF-2α is approximately 50% homologous to HIF-1α at the amino acid level, their distributions, target genes, and functions are notably different[26–28]. HIF-1α is expressed mainly in hypoxic, poorly vascularized sites, while HIF-2α is also expressed in well-vascularized tissues[27,28].

In cartilage as well, the hypoxic condition enhances the expression and activity of HIF-1α[24,29–31]. HIF-1α is extensively localized in chondrocytes from the earlier differentiation stages[21,29–31], and induces initial chondrogenesis, joint development, cartilage matrix synthesis, and cell survival[29–33]. 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 cartilage[21,34,35]. Likewise, although cartilage-specific knockout of HIF-1α leads to defects in cartilage formation and chondrocyte survival[31,33], HIF-2α-haploinsufficient mice exhibit mild and transient dwarfism 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 factor-2 (RUNX2), Indian hedgehog (IHH), and vascular endothelial growth factor (VEGF)[21]. Hence, HIF-1α and HIF-2α may have distinct roles *via* different mechanisms: hypoxia-dependent cartilage formation and maintenance by HIF-1α and hypoxia-independent endochondral ossification by HIF-2α.

HIF β-subunit partner of HIF-2α

The HIF α-subunit member functions by forming a heterodimer with a β-subunit member[22,23]. The representative β-subunit is the aryl hydrocarbon nuclear translocator (ARNT or HIF-1β), previously identified as a binding partner of the dioxin/aryl hydrocarbon receptor (DR/AhR)[36]. ARNT-like (ARNTL, BMAL1 or MOP3) is well characterized as a regulator of circadian rhythms, working as a heterodimer with CLOCK[37]. ARNT2 (HIF-2β) and ARNTL2 (BMAL2 or CLIF) are identified as homologs of ARNT and ARNTL, respectively[38–40]. In the mouse cartilage, while ARNT and ARNT2 are extensively expressed in chondrocytes of all differentiation stages, ARNTL and ARNTL2 are expressed in highly differentiated chondrocytes, just like HIF-2α[21]. Although all β-subunit members are physically associated with HIF-2α as previously reported[40–43], ARNTL shows the strongest binding affinity to HIF-2α and enhancement of the HIF-2α transactivation of *COL10A1*, *MMP13* and *VEGFA*[21]. Meanwhile, ARNTL-null mice show impaired circadian rhythms and ectopic ossification with age without abnormality in skeletal development or joint cartilage[44]. 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 development[21,35]. 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]. 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 factors[35]. Furthermore, human genomic analyses using a Japanese population-based cohort of the ROAD study[45] have identified a functional single nucleotide polymorphism (SNP) in the human HIF-2α gene which is associated with knee OA[21].

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 cartilage[21,35]. Because MMP-13-null mice are reported to be protected from cartilage degradation after surgical OA induction[4], 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 development[3,5,46,47], ADAMTS-5 is not altered by the over-expression or knockout of HIF-2α, implicating an independent pathway[21,35]. Besides catabolic factors, HIF-2α induces osteogenic factors like RUNX2, IHH and VEGF[21]. RUNX2 is a mediator of chondrocyte hypertrophy and of MMP-13 expression just weeks into the course of the experimental mouse knee OA model[14]. 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 functions[21]. The IHH signal is also shown to promote OA development as well as the physiological skeletal growth, although being partly mediated by RUNX2 expression[47]. VEGF is a well-characterized growth factor inducing angiogenesis, and a representative target gene of HIF-1α[31,48]. VEGF may be a major mediator for the osteophyte formation induced by HIF-2α, since this is highly expressed in the periphery of OA cartilage[21].

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 chondrocytes[21,35]. 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 analysis[21]. 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

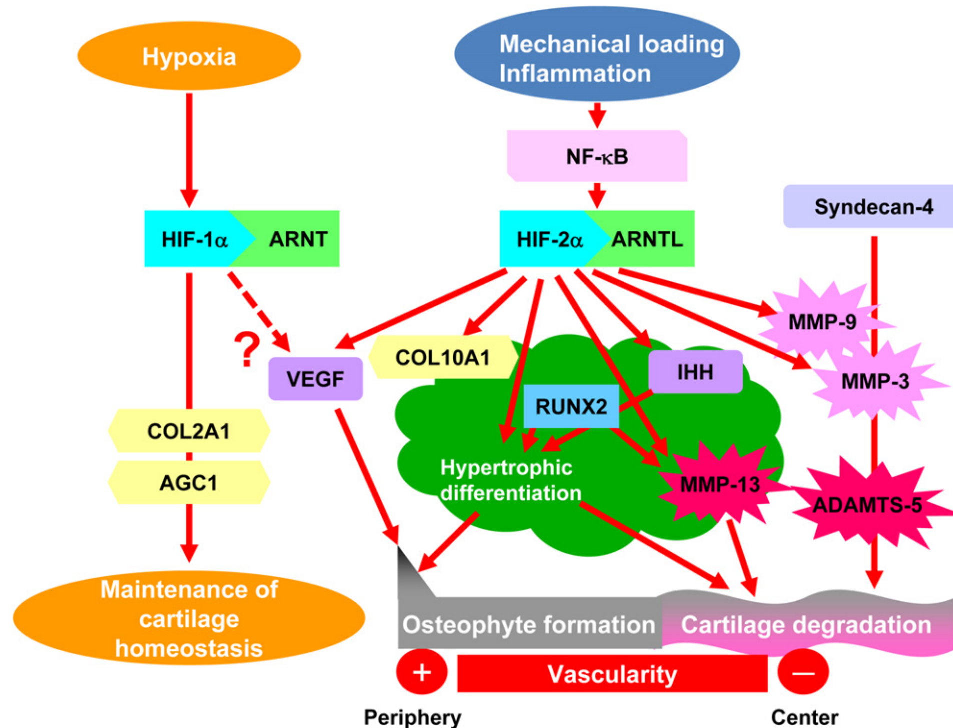

Fig. 1. Schematic overview of signal transductions on the axis of HIF proteins in the maintenance of cartilage homeostasis and OA development.

mechanical stress[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[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[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[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 transactivation 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[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[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[21] might be useful for the switching from HIF-2α to HIF-1α. Further systems biology of chondrocyte differentiation and potential regulatory effects of chondrocyte microRNAs[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.

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