

# Hypoxia-Inducible Factors (HIFs) in the articular cartilage: a systematic review

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**Abstract.** – Osteoarthritis (OA) is the most common joint disease, and in recent years has become a major public health problem. The hallmark of OA is cartilage destruction with local commitment of subchondral bone and the synovial membrane. Hypoxia-inducible factors (HIFs) are transcriptional factors and key regulators of the cellular response to hypoxia. To date, three members of the human HIF- $\alpha$  protein family have been described: HIF-1 $\alpha$ , HIF-2 $\alpha$ , and HIF-3 $\alpha$ . HIF-1 $\alpha$  plays an essential role in the articular cartilage (a hypoxic tissue), as it has a protective effect in the maintenance of the articular cartilage matrix, HIF-2 $\alpha$  has a harmful effect on the articular cartilage matrix, and HIF-3 $\alpha$  acts as a negative regulator of HIF-1 $\alpha$  and HIF-2 $\alpha$ . Due to the recent growing interest in the role of HIFs in rheumatic diseases, we focused this review on the potential role of these key regulators in articular cartilage maintenance as the central axis in OA development.

*Key Words:*

Osteoarthritis, HIF-1 $\alpha$ , HIF-2 $\alpha$ , HIF-3 $\alpha$ , Hypoxia in articular cartilage.

## Introduction

Osteoarthritis (OA) is the most common joint disease, and in recent years has become an important public health problem. The hallmark of OA is cartilage destruction with local commitment of subchondral bone and the synovial membrane. Changes in the articular cartilage, subchondral bone, and synovial membrane are mediated by the cells present in those three compartments, i.e. chondrocytes, cells of osteoblast lineage, and synoviocytes, respectively, whose primary role is to maintain the integrity and function of these tissues<sup>1,2</sup>. Articular cartilage is a connective avascular, aneural, and aliphatic tissue that works

hydrodynamically to support and distribute the load of the body and provide an almost friction-free movement in joints<sup>3</sup>. Cartilage structure consists of mainly water (60%-80%), and chondrocytes (4%) are the only cell type present. The remaining part of this tissue is made of collagen, proteoglycans, glycoproteins, and lipids<sup>4</sup>. Since articular cartilage has no capillary networks, the cartilage microenvironment is hypoxic. Hypoxia is a state where oxygen availability/delivery is below the level required to maintain physiological oxygen tension in a particular tissue<sup>5</sup>. In this regard, it is known that under healthy conditions, oxygen concentration in articular cartilage varies from 0.5% to 10% (4-70 mmHg, respectively)<sup>6,7</sup>. It is well established that when tissue oxygen demand exceeds supply, a cascade of intracellular events is activated increasing expression of hypoxia-inducible factors (HIFs). As a consequence, the extensive transcriptional response regulating angiogenesis, glucose metabolism, cell growth, metastasis, and others processes, is induced<sup>8</sup>. Due to the recent growing interest in the involvement of HIFs in rheumatic diseases, particularly in OA<sup>9</sup>, we focused this review on the potential role of HIFs in the maintenance of healthy articular cartilage as the central axis in the development of this pathology.

## Methods

### *Search Criteria and Selection Process*

All relevant literature in the field of HIFs in connection with OA published in the last 16 years was reviewed. We included original articles about studies in humans and animals, published between January 2000 and May 2016. To identify all available studies, a detailed search on

the topic of this review was conducted according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines<sup>10</sup>. A systematic search was performed in the electronic databases PubMed and Embase using the following Medical Subject Headings (MeSH) search terms in all possible combinations: “hypoxia inducible factor 1 alpha”, “hypoxia inducible factor 2 alpha”, “hypoxia inducible factor 3 alpha”, “osteoarthritis”, “articular cartilage”, and “hypoxia in cartilage”. Also, the reference lists of all retrieved articles were manually reviewed. In the case of missing data, study authors were contacted to try to retrieve the original data. Two authors (JFT and YZC) analyzed each article and extracted the data independently. In the case of disagreement, a third and fourth researchers were consulted (GMN and ALR). Discrepancies were resolved by consensus.

### ***Inclusion and Exclusion Criteria***

The following types of publications were excluded: articles not published in English, case reports, and letters to the editor. The search results were filtered to avoid duplicates. Titles, abstracts, and full reports of the articles identified were systematically filtered using inclusion and exclusion criteria. Given the properties of the studies involved, the methodological quality of each study was evaluated with the Newcastle–Ottawa Scale (NOS), which was specifically developed to assess the quality of non-randomized observational studies<sup>11</sup>.

## **Results**

Approximately 8887 publications were identified in the PubMed and Embase databases between January 2000 and June 2016, but only 63 full-text articles were assessed as eligible for the study. The results of the search strategy are illustrated in Figure 1.

## **Discussion**

### ***Three Structurally Similar Molecules That Have Different Functions Within Articular Cartilage***

HIFs are heterodimers consisting of two subunits: the unstable hypoxia-regulated  $\alpha$  subunit (HIF- $\alpha$ ) and the stable oxygen-insensitive  $\beta$  subunit (HIF- $\beta$ ), which is also known as an

aryl-hydrocarbon receptor nuclear translocator (ARNT)<sup>12</sup>. Both HIF- $\alpha$  and HIF- $\beta$  belong to the basic helix-loop-helix (bHLH)-Per-Arnt-Sim (PAS) family of transcription factors, which share several conserved structural domains, including a bHLH region for DNA binding and two PAS domains for target gene specificity and dimerization<sup>13,14</sup>. Figure 2 illustrates the structure of HIFs, and Figure 3 shows HIF activity under normoxia and hypoxia conditions. To date, three members of the human HIFs- $\alpha$  family have been described: HIF-1 $\alpha$ , HIF-2 $\alpha$  and HIF-3 $\alpha$ . Due to the hypoxic conditions in articular cartilage, the three members of the HIF- $\alpha$  protein family play an essential role: HIF-1 $\alpha$  has a beneficial effect in the maintenance of the articular cartilage matrix, HIF-2 $\alpha$  has a harmful effect on the articular cartilage matrix, and HIF-3 $\alpha$  acts as dominant-negative regulator of HIF-1 $\alpha$  and HIF-2 $\alpha$ <sup>15</sup>.

### ***HIF-1 $\alpha$***

In 1995, Wang and Semenza<sup>16</sup> purified and characterized a protein of 120 kDa, which was subsequently named HIF-1 $\alpha$ . HIF-1 $\alpha$  is an 826-amino-acid polypeptide encoded by the HIF1A gene located in chromosome 14 (14q23.2), and plays a critical role in oxygen homeostasis in chondrocytes and other cell types<sup>17,18</sup>. HIF-1 $\alpha$  is the master of transcription factors and acts as a physiological regulator. In normoxic conditions, HIF-1 $\alpha$  is hydroxylated on key proline residues (Pro402/564) within the oxygen-dependent degradation (ODD) domain by prolyl hydroxylases (PHDs), which are dioxygenases dependent of oxygen, Fe<sup>2+</sup>, ascorbic acid, and 2-oxoglutarate. This hydroxylation allows recognition of HIF-1 $\alpha$  by the von Hippel-Lindau (pVHL) tumor-suppressor protein, the substrate recognition component of an E3 ubiquitin ligase complex that targets HIF- $\alpha$  for proteasomal degradation<sup>12,18,19</sup>. In this sense, the ODD domain functions as a true oxygen sensor. Another oxygen sensor is factor inhibiting HIF-1 (FIH-1), which hydroxylates HIF-1 $\alpha$  in the presence of oxygen in the asparagine residue 803 within the C-terminal transcriptional activation domain (C-TAD), and which remains inactive in hypoxia, allowing the interaction of HIF-1 $\alpha$  with the coactivators CBP and p300. On the other hand, under hypoxic conditions, HIF-1 $\alpha$  hydroxylation is inhibited and it accumulates in the cytoplasm, allowing its phosphorylation and subsequent translocation to the nucleus, where it dimerizes with HIF- $\beta$  and it binds (as a heterodimer) to hypoxia response

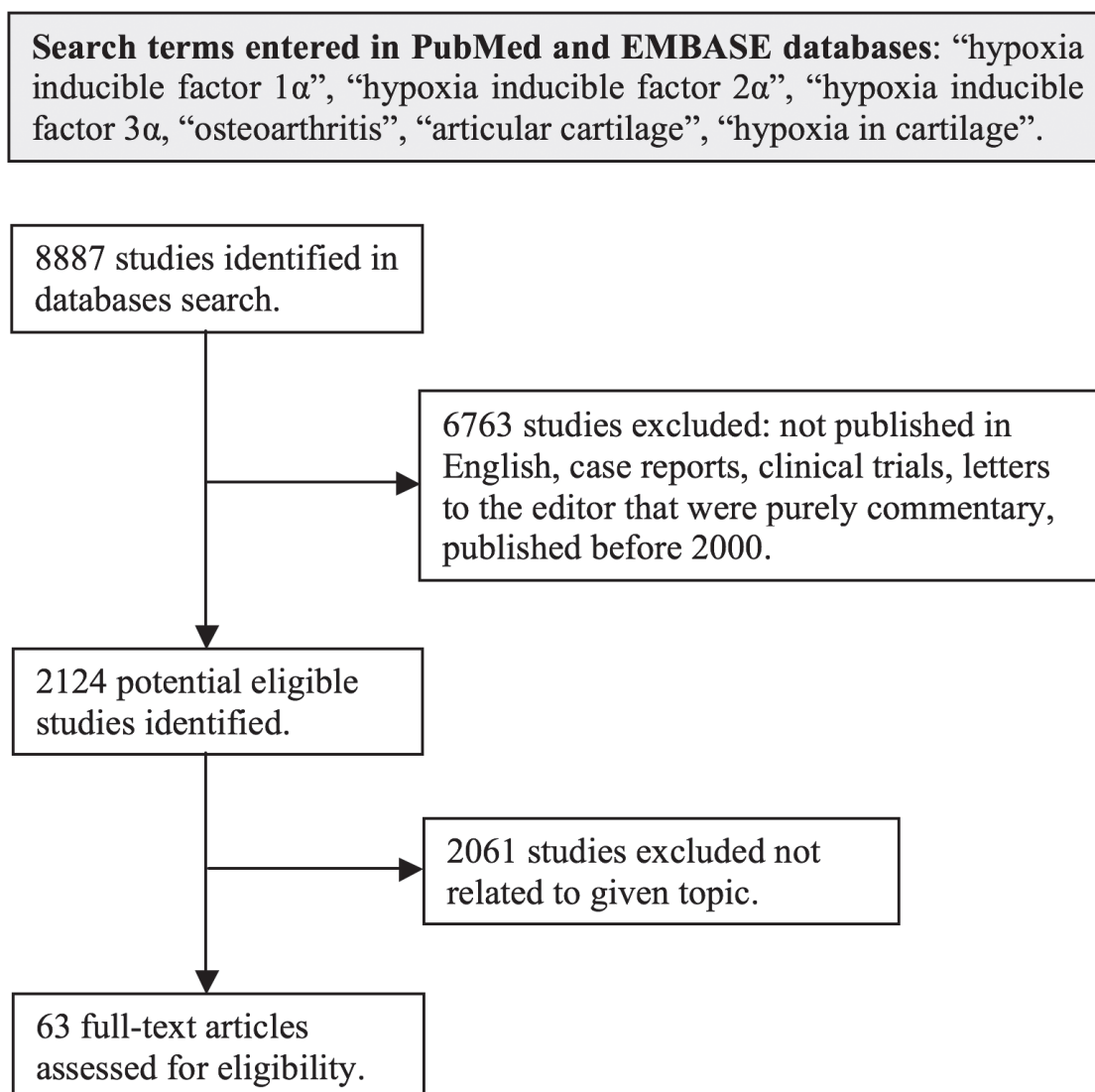
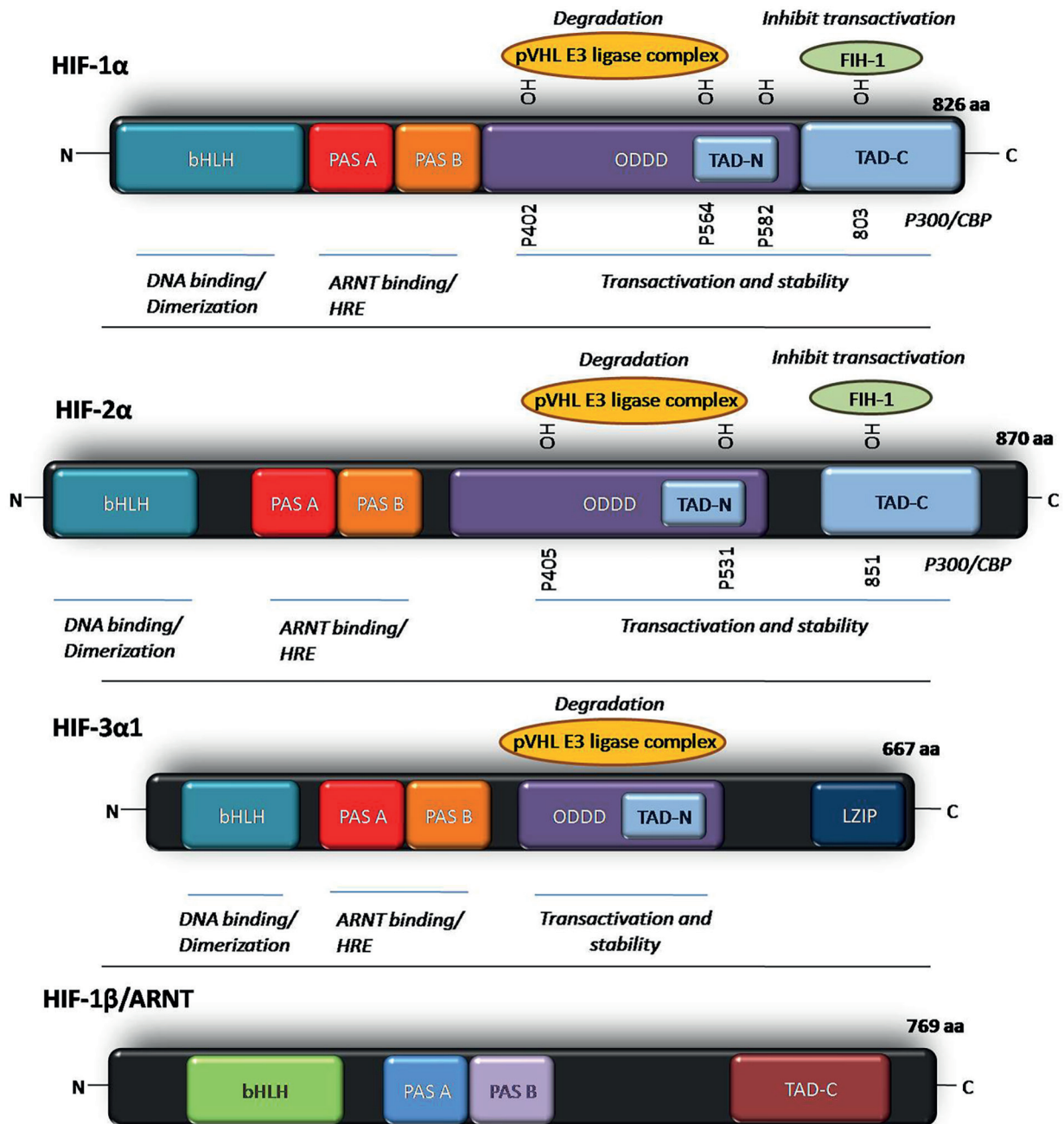


Figure 1. Representation of the search strategy.

elements (HREs) on hypoxia-sensitive genes, such as NOS2, VEGF, EPO, GLUT1, SOX9, IGF2, COL2A1, among many others<sup>20,21</sup>. The transcription of such target genes regulated by HIF-1 $\alpha$  might potentially maintain the chondroprotective functions that are compromised by the detrimental conditions in the damaged joint environment<sup>6,22,23</sup>. Gelse et al<sup>24</sup> investigated the effects of HIF-1 $\alpha$  inhibition and stabilization by 2-methoxyestradiol (2ME2) and dimethylglyoxal (DMOG), respectively, on OA progression in murine knee joints. Likewise, Grimmer et al<sup>25</sup> demonstrated that under hypoxic conditions, primary human articular chondrocytes enhanced accumulation of native type II collagen and stabilized HIF-1 $\alpha$ , but the effect

was suppressed by adding 2ME2. These two reports show the importance of HIF-1 $\alpha$  in maintaining the integrity of the extracellular matrix of articular cartilage. Meanwhile, Sakamoto et al<sup>26</sup> investigated the roles of HIF-1 $\alpha$ , the vascular endothelial growth factor (VEGF), and the anti-angiogenic factor chondromodulin-1 (ChM-1) in cartilage degeneration in immobilized rat joints. They found that immobilization induces thinning of the articular cartilage and its degeneration, and the appearance of a vascular channel in areas with balanced expression of HIF-1 $\alpha$ /VEGF and ChM-1. Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), the most abundant prostaglandin in the skeletal system, induces some matrix-degrading enzymes, thus stimulating the catabolism of cartilage, but

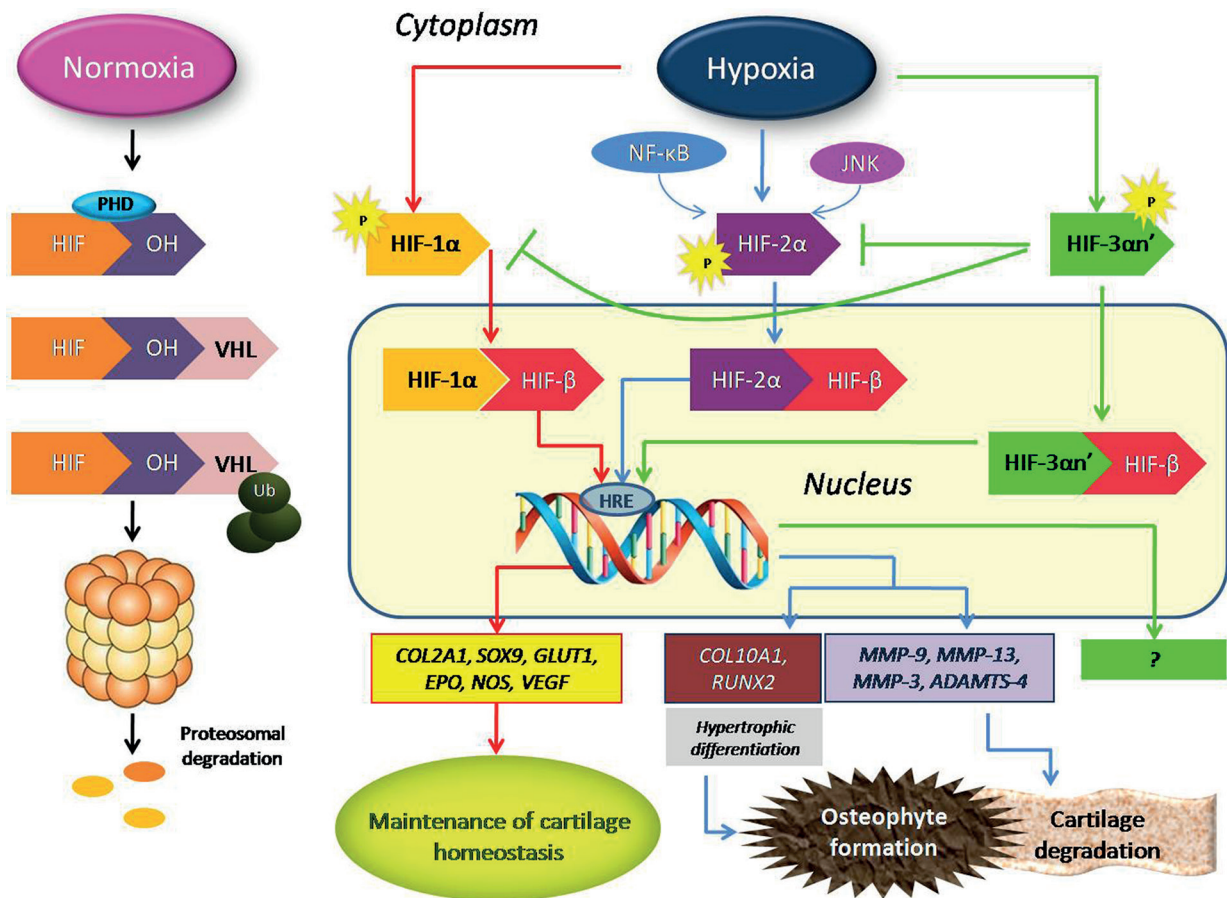


**Figure 2.** Schematic overview of the domain structures of hypoxia-inducible factor (HIF) members. The structural domains of HIF-1 $\alpha$ , -2 $\alpha$ , -3 $\alpha$  and their transcriptional binding partner, HIF-1 $\beta$ /ARNT (aryl hydrocarbon nuclear translocator), which together form the [HIF- $\alpha$ /HIF-1 $\beta$ ] transcriptional complexes. The NH<sub>2</sub>-terminal of HIF- $\alpha$  and HIF-1 $\beta$  consists of bHLH (basic helix-loop-helix) and PAS (Per-ARNT-Sim homology) domains that are required for heterodimerization and DNA binding in the hypoxia response elements (HRE) at the target gene loci. The COOH-terminal of HIF- $\alpha$  contains two transactivation domains (TADs). The short half-life of HIF- $\alpha$  under nonhypoxic conditions is due to fast ubiquitination and proteasomal degradation. HIF-1 $\alpha$ , -2 $\alpha$ , -3 $\alpha$  also contain an oxygen-dependent degradation (ODD) domain, which contains the conserved proline(s). Only HIF-3 $\alpha$  contains a leucine zipper (LZIP) domain in the COOH-terminal region; also, it differs from HIF-1 $\alpha$  and HIF-2 $\alpha$  in its lack of the TAD-C domain.

it also plays a role in cartilage anabolism. The synthesis of PGE<sub>2</sub> is the end point of a sequence of enzymatic reactions involving phospholipase A<sub>2</sub>, cyclooxygenase 2 (COX-2), and microsomal

prostaglandin E synthase 1 (mPGES-1). Grimmer et al<sup>27</sup> and Claveau et al<sup>28</sup> studied the catabolic and anabolic pathways of OA and healthy cartilage by examining the synthesis and dis-





**Figure 3.** Hypoxia-inducible factors (HIFs) activity under normoxia and hypoxia conditions. Under normoxia conditions, the specific proline residues on the ODD domain for each HIF member are hydroxylated by oxygen-dependent prolyl-hydroxylases (PHDs) that lead to the formation of a complex with the von Hippel-Lindau (VHL) factor, which in turn, binds to ubiquitin (Ub) and is subsequently degraded by the proteasome. However, under hypoxic conditions, HIFs are stabilized and phosphorylated by mitogen-activated protein kinase (MAPK); once phosphorylated, HIFs translocate to the nucleus and bind to the HIF-1 $\beta$  subunit to form the [HIF- $\alpha$ /HIF-1 $\beta$ ] complex. This complex through the HRE binds to a specific DNA sequence (5'TAGCGTGH3') in the promoter regions of several genes for their subsequent activation. The n represents the three isoform of HIF-3 $\alpha$ . Taken and modified from Saito et al<sup>38</sup>.

tribution pattern of mPGES-1, and determined that HIF-1 $\alpha$  is involved in the up-regulation of mPGES-1 and may, therefore, plays an important role in the cartilage metabolism of OA patients. It has also been reported that HIF-1 $\alpha$  participates in the multistep pathway of mesenchymal cell differentiation into chondrocytes. The SOX9 gene is expressed in all chondrocyte progenitors and chondrocytes, but its expression is completely turned off in hypertrophic chondrocytes. In the study conducted by Zhang et al<sup>29</sup>, they demonstrated that HIF-1 $\alpha$  is a positive regulator of SOX9, which is required for chondrocyte differentiation and cartilage formation. Finally, another aspect that is worth mentioning is the fact that genetic variants in the HIF1A gene can alter the expression of the HIF-1 $\alpha$  protein, and even its

stability. Tanimoto et al<sup>30</sup> tested the rs11549465 and rs11549467 single nucleotide polymorphisms (SNP) of the HIF1A gene in patients with primary head and neck squamous cell carcinoma; both HIF1A variants showed a significantly higher transcription activity than the wild-type under normoxic and hypoxic conditions ( $p < 0.02$ ). Meanwhile, Yamada et al<sup>31</sup> evaluated the rs11549465 polymorphism in patients with type 2 diabetes, and determined that there was an association ( $p=0.003$ ). Recently, our group carried out a case-control study that evaluated the presence of the rs11549465 polymorphism in patients with knee OA, and concluded that it could be playing a protective role in the loss of articular cartilage (CT genotype or T allele, OR = 0.2;  $p = 0.003$  and  $p = 0.004$ , respectively)<sup>32</sup>.

**HIF-2 $\alpha$** 

The second member of the HIF- $\alpha$  subunit, HIF-2 $\alpha$ , was identified in 1997<sup>33</sup>. This transcription factor is an 870-amino-acid polypeptide and is also known as endothelial PAS domain protein 1 (Epas1). In humans, it is encoded by the EPAS1 gene located in chromosome 2. The variability between HIF-1 $\alpha$  and HIF-2 $\alpha$  is observed mostly within the N-terminal transactivation domain (N-TAD). Whereas HIF-1 $\alpha$  preferentially induces glycolytic pathways, HIF-2 $\alpha$  regulates important genes involved in tumor growth, cell cycle progression, and maintenance of stem cell pluripotency. Similarly to HIF-1 $\alpha$ , the ODD domain of HIF-2 $\alpha$  is positioned in Pro405 and Pro531<sup>5,8,34</sup>. In 2004, Coimbra et al<sup>35</sup> demonstrated for the first time the expression of HIF-2 $\alpha$  mRNA in both normal and OA human articular chondrocytes. This finding opened the possibility for a more thorough analysis of the involvement of HIF-2 $\alpha$  in the osteoarthritic process. Later on, Ryu et al<sup>36</sup> proved that HIF-2 $\alpha$  is a catabolic regulator of cartilage destruction that acts by regulating the expression of various catabolic factors, including matrix-degrading enzymes and inflammation mediators. They had previously demonstrated that IL-6 acts as a crucial mediator of HIF-2 $\alpha$ -induced experimental OA cartilage destruction in mice via regulation of metalloproteinases MMP3 and MMP13 levels<sup>37</sup>. In addition, it has been proposed that NF- $\kappa$ B signaling is an upstream mechanism that regulates HIF-2 $\alpha$ , since IL-1 $\beta$  and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), putative ligands for the NF- $\kappa$ B signaling pathway, increase HIF-2 $\alpha$  expression in chondrocytes<sup>38,39</sup>. There is evidence suggesting that HIF-2 $\alpha$  participates in endochondral ossification, which plays a crucial role in OA as well as in physiological skeletal growth. In this regard, it has been observed that HIF-2 $\alpha$  enhances transactivation of COL10A1, MMP13, and VEGF through specific binding to the respective HREs<sup>40-42</sup>. Additionally, Saito et al<sup>40</sup> and Yang et al<sup>43</sup> found independently that genetic deletion of one HIF-2 $\alpha$  allele (Epas1 $+/$ -mice) lessened the severity of experimentally induced OA. They also observed massive joint tissue destruction following local injection of Adenovirus-Epas1 virus into mouse knees. Furthermore, they also studied mice overexpressing Epas1 in a tissue-specific manner, and reported spontaneous cartilage destruction in aged mice. Numerous inflammatory cytokines, such as IL-1, IL-6, and TNF $\alpha$ , take part in the

pathogenesis of OA, increasing the expression of proteolytic enzymes and MMPs, and accelerating cartilage destruction. Sartori-Cintra et al<sup>44</sup> analyzed the regulation of HIF-1 $\alpha$  by IL-1 $\beta$ , and they found that IL-1 $\beta$  up-regulated the levels of the HIF-1 $\alpha$  protein post-transcriptionally in human OA chondrocytes. Meanwhile, Yang et al<sup>43</sup> found that HIF-2 $\alpha$  was induced in mouse articular chondrocytes upon treatment with IL-1 $\beta$ . In contrast, Yudoh et al<sup>45</sup> observed that the HIF-2 $\alpha$  protein levels are not affected by IL-1 $\beta$  treatment in human articular chondrocytes, whereas the HIF-1 $\alpha$  protein is indeed strongly increased. From the genetic point of view, the presence of SNP might influence HIF-2 $\alpha$  regulation. Nakajima et al<sup>46</sup> evaluated the association of rs17039192 SNP in knee OA patients through a meta-analysis from six countries and showed that there is a negative association between the HIF-2 $\alpha$  rs17039192 polymorphism risk allele and knee OA susceptibility (OR < 1). In another aspect, Bohensky et al<sup>47</sup> performed a study focused on the regulation of autophagy in human and murine cartilage, and observed that HIF-1 $\alpha$  responds to the tissue oxemic state and promotes chondrocyte autophagy, but HIF-2 $\alpha$  behaved as a potent negative regulator of autophagy in maturing chondrocytes. They suggest that the HIF-2 $\alpha$  protein acts as a brake of the autophagy-accelerator function of HIF-1 $\alpha$ . In summary, HIF-2 $\alpha$  apparently proves to be the “villain” in OA. The results mentioned above indicate that HIF-2 $\alpha$  is a molecule structurally and functionally similar to HIF-1 $\alpha$ , but with different target genes. Whereas HIF-1 $\alpha$  induces expression of COL2A1, SOX9, GLUT1, EPO, NOS, and VEGF in order to maintain cartilage homeostasis, HIF-2 $\alpha$  induces expression of genes associated with hypertrophic differentiation, such as COL10A1 and RUNX2 (osteophyte formation), and genes associated with cartilage degradation, such as MMP9, MMP13, MMP3, ADAMTS<sup>48-51</sup>.

**HIF-3 $\alpha$** 

In 1998, Gu et al<sup>52</sup> identified another HIF- $\alpha$  isoform in mice sharing a ~55% amino acid sequence identity with HIF-1 $\alpha$  and HIF-2 $\alpha$  in its bHLH PAS domain, which was categorized as the third member of the HIF transcription factor family: HIF-3 $\alpha$ <sup>53</sup>. However, to date, the regulation mechanism of HIF-3 $\alpha$  levels within cells by hypoxia is still poorly understood<sup>54</sup>. Human HIF-3 $\alpha$  was identified in 2001 by Hara et al<sup>33</sup> and is located at the 19q13.13-13.2 locus;

its open reading frame encodes a 662-amino acid protein with a predicted molecular weight of 73 kDa. HIF-3 $\alpha$  differs from HIF-1 $\alpha$  and HIF-2 $\alpha$  in protein structure and regulation of gene expression. For a long time, HIF-3 $\alpha$  was considered a negative mediator of HIF-regulated genes, since it also has a transcriptional regulatory function; however, it affects gene expression negatively by competing against HIF-1 $\alpha$  and HIF-2 $\alpha$  for binding to their transcriptional elements in target genes during hypoxia<sup>55-57</sup>. Drevytska et al<sup>58</sup> analyzed the mRNA expression of HIFs in different tissues of an animal model and found that under acute hypoxia, the HIF-3 $\alpha$  subunit is essential for the hypoxic response, but it plays a negative role in the adaptation to hypoxia. HIF-2 $\alpha$  and HIF-3 $\alpha$  appear to be expressed in a cell-specific manner when compared to the ubiquitously expressed HIF-1 $\alpha$ <sup>59</sup>. The human HIF-3 $\alpha$  locus gives rise to multiple HIF-3 $\alpha$  isoforms due to diverse promoters, different transcription initiation sites, and alternative splicing<sup>57</sup>. Recent studies suggest that HIF-3 $\alpha$  isoforms have different and even opposite functions. Many HIF-3 $\alpha$  target genes have been identified, such as IGFBP-1, EPO, GLUT1, ANGPTL4, SOX2, and FOXP2<sup>60</sup>. While some short HIF-3 $\alpha$  isoforms act as dominant-negative regulators of HIF-1 $\alpha$ /2 $\alpha$ , other HIF-3 $\alpha$  isoforms can inhibit HIF-1 $\alpha$ /2 $\alpha$  function by competing for the HIF- $\beta$  subunit<sup>60-62</sup>. HIF-3 $\alpha$  was first described in chondrocytes in the study performed by Schrobback et al<sup>63</sup> in 2012. They found that chondrocytes cultured under normoxic and hypoxic conditions expressed HIF-3 $\alpha$ . Although the mRNA transcript levels were extremely low, which could potentially mean that it is only expressed by a minority of chondrocytes, its transcription was dependent on low oxygen concentrations and seemed to follow the expression profiles of other chondrogenic marker genes during differentiation. Troponin I type 2 (TNNI2) encodes a fast-twitch skeletal muscle protein. Zhu et al<sup>64</sup> evaluated the effect of TNNI2 on HIF-3 $\alpha$  regulation in an *in vivo* study, and showed that the TNNI2 protein binds to the HIF-3 $\alpha$  promoter in primary osteoblasts. Knock-in mice carrying a mutant TNNI2 showed increased HIF-3 $\alpha$  expression in the long bone. The increased amount of HIF-3 $\alpha$  was linked to impairment of angiogenesis, delay in endochondral ossification, and decreased chondrocyte differentiation and osteoblast proliferation. Markway et al<sup>65</sup> characterized HIF-3 $\alpha$  expression during chondrocyte differentiation *in vitro* in cartilage tissues. Their results indicate

that HIF-3 $\alpha$  levels are suggestive of the hypertrophic state of chondrogenic cells, and one or more splice variants may be important regulators of the chondrocyte phenotype.

### ***Therapeutic Approach of HIFs in OA***

Articular cartilage has a very limited restorative capacity. Once articular cartilage is damaged, full recovery of its structure, function, and biomechanical properties is rarely expected in most cases; the damaged articular cartilage potentially suffers continuous degeneration<sup>66</sup>. There have been several studies covering the therapeutic aspects in OA, but they have not proved to be completely effective. Loss of articular cartilage is an irreversible process of OA, and HIFs play an important role in the process, which suggests the possibility of these molecules being a central point for a new potential therapeutic approach for the disease.

### ***PHD Inhibitors***

Under normoxia conditions, certain conserved proline residues (Pro-402 and -564 in HIF-1 $\alpha$ , Pro-405 and -531 in HIF-2 $\alpha$ , and Pro-490 in HIF-3 $\alpha$ ) within the ODD domain are hydroxylated by PHD proteins. PHDs are members of a large class of enzymes known as 2-oxoglutarate-dependent dioxygenases, which catalyze the incorporation of oxygen into organic substrates through a mechanism that requires 2-oxoglutarate ( $\alpha$ -ketoglutarate), iron (Fe<sup>2+</sup>), and ascorbate. There are four types of PHDs: PHD1, PHD2, PHD3, and PHD4<sup>67,68</sup>. After the hydroxylation process, HIFs are ubiquitinated and degraded in the proteasome. Based on this aspect and with the information presented above, stabilizing HIF-1 $\alpha$  through PHD inhibition could prove to have a potential therapeutic value. There is evidence that endogenous HIF-1 $\alpha$  levels can be increased through suppression of PHD activity, either by reducing the cellular oxygen level or by combining the Fe<sup>2+</sup> competitively. DMOG is a cell-permeable competitive inhibitor of PHDs, and an analogue of 2-oxoglutarate; in this way, it inhibits not only the HIF prolyl, but also asparaginyl hydroxylases<sup>69</sup>. Recently, two novel PHD inhibitors, TM6008 and TM6089, were synthesized based on the three-dimensional protein structure of human PHD2. Both selectively inhibit PHD2 by binding to its active site (where HIFs bind to it), stimulating HIF activity. On the other hand, the FK506-binding protein 38 (FKBP38) could also be used as a PHD2



inhibitor, since it has been demonstrated that it decreases PHD2 protein stability through interaction with the N-terminal domain of PHD2<sup>70</sup>. Thirunavukkarasu et al<sup>71</sup> carried out a study in diabetic rats and they observed that PHD3 levels decrease when simvastatin is applied after myocardial infarction; surprisingly, HIF-1 $\alpha$  levels increased in comparison to the control group. However, the effect of simvastatin in articular cartilage must be tested and determined. Other inhibitor of PHDs that should be evaluated with therapeutic potential for articular maintenance is desferrioxamine (DFO). DFO is a small molecule that inhibits prolyl hydroxylation of HIF, and stabilized HIF/VEGF production. Wan et al<sup>72</sup> applied DFO in a model *in vivo* and showed an increase of angiogenesis levels and markedly improved bone regeneration.

### HIF-2 $\alpha$ Inhibitors

A promising therapeutic strategy is the specific inhibition of HIF-2 $\alpha$ . One solution to this challenge might be the intra-articular injection of the HIF-2 $\alpha$  inhibitor. Although joint cartilage cells would take up the injected inhibitor via diffusion from synovial fluid and the surrounding joint tissue, transcription factors are still difficult to target<sup>38</sup>. Recently, Li et al<sup>73</sup> performed an *in vivo* study with intra-articular injections of resveratrol as inhibitor of HIF-2 $\alpha$  in mice. They found that resveratrol prevents OA progression by activating the silent information regulator 2 type 1 (SIRT1), thereby silencing HIF-2 $\alpha$ . SIRT1 is crucial for inhibiting NF- $\kappa$ B and subsequently down-regulates NF- $\kappa$ B-induced catabolism-related genes, such as COX2, IL-1 $\beta$ , and IL-6, which play important roles in OA pathogenesis. Finally, the YC-1 [13 $\alpha$ -3-(50-hydroxymethyl-20-furyl)-1-benzylindazole] molecule was originally described as an inhibitor of platelet aggregation and as a vasodilator. A new biological function was also found: inhibition of HIF-2 $\alpha$  activity<sup>74</sup>. There are other treatment options, such as cell therapy and tissue engineering, which are still under research and are currently offering encouraging results<sup>66,75,76</sup>. Other therapies that may be of interest in terms of repairing cartilage include the application of hyperbaric oxygen (HBO<sub>2</sub>), which has been shown to exert a trophic effect on vasculogenic stem cells (SC) in an animal model. HBO<sub>2</sub>-treated SC exhibited higher levels of thioredoxin-1 (Trx1) and Trx1 reductase, as well as higher levels of HIF-1 $\alpha$ , -2 $\alpha$ , and -3 $\alpha$ , and other HIF-1 $\alpha$ -dependent growth factors<sup>77</sup>.

### Conclusions

The study of hypoxia-inducible factors in articular cartilage opens a new field of research in their physiology, metabolism, and therapeutic targets. Cartilage destruction in OA is mediated by catabolic enzymes and chondrocyte death, including apoptosis and/or autophagy; all these processes contribute greatly to the pathogenesis. The information presented above shows that the expression of HIFs is increased in OA cartilage. HIFs are three structurally similar molecules with different functions within the articular cartilage. HIF-1 $\alpha$  acts as a survival factor by enhancing extracellular matrix synthesis and inhibiting apoptosis, and is of pivotal importance in cartilage homeostasis. HIF-2 $\alpha$  is a catabolic regulator of cartilage destruction that acts by regulating matrix-degrading enzymes and inflammation mediators. The HIF-2 $\alpha$  protein act as a brake for the autophagy-accelerator function of HIF-1 $\alpha$  and promotes chondrocyte hypertrophy characterized by a unique gene expression program, including type X collagen and the type II collagen-degrading protease MMP-13. Last, but not least, HIF-3 $\alpha$  has a transcriptional regulatory function, which negatively affects gene expression by competing against HIF-1 $\alpha$  and HIF-2 $\alpha$  to bind to transcriptional elements in target genes during hypoxia. Although HIF-3 $\alpha$  has alternative splicing variants, the specific functions of any of them have not been fully determined yet. Various therapeutic mechanisms for OA based on HIF signaling have been proposed; however, further studies should explain the exact mechanism of HIFs in OA development and progression.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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