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Review article

Role of HIF-1α signaling pathway in osteoarthritis: a systematic review

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ABSTRACT

Osteoarthritis (OA) is the most common form of arthritis and is frequently diagnosed and managed in primary care; it is characterized by loss of articular hyaline cartilage, which is a unique connective tissue that physiologically lacks blood vessels. Articular cartilage survives in a microenvironment devoid of oxygen, which is regulated by hypoxia inducible factor (HIF-1α). HIF-1α is considered the main transcriptional regulator of cellular and developmental response to hypoxia. To date, the relevance of HIF-1α in the assessment of cartilage has increased since its participation is essential in the homeostasis of this tissue. Taking into account the new emerging insights of HIF-1α in the scientific literature in the last years, we focused the present review on the potential role of HIF-1α signaling pathway in OA development, especially in how some genetic factors may influence the maintenance or breakdown of articular cartilage.

Papel da via de sinalização do HIF-1α na osteoartrite: revisão sistemática

Palavras-chave:
Fator induzível por hipóxia 1-α
Via de sinalização do HIF-1α
Polimorfismos genéticos
Osteoartrite

RESUMO

A osteoartrite (OA) é a forma mais comum de artrite e frequentemente é diagnosticada e gerenciada na atenção primária; é caracterizada por perda da cartilagem articular hialina, um tecido conjuntivo único que fisiologicamente carece de vasos sanguíneos. A cartilagem articular sobrevive em um microambiente desprovido de oxigênio, que é regulado pelo fator induzível por hipóxia-1α (HIF-1α). O HIF-1α é considerado o principal regulador transcricional da resposta celular e de desenvolvimento à hipóxia. Na atualidade, a relevância

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do HIF-1α na avaliação da cartilagem tem aumentado, já que a sua participação é essencial na homeostase desse tecido. Considerando as novas perspectivas emergentes do HIF-1α na literatura científica nos últimos anos, foca-se a presente revisão no potencial papel da via de sinalização do HIF-1α no desenvolvimento da OA, especialmente no modo como alguns fatores genéticos podem influenciar na manutenção ou ruptura da cartilagem articular.

Introduction

Osteoarthritis (OA) is a common chronic condition affecting millions of people worldwide and is a considerable cause of disability.[1] It is the most common rheumatic disease, every age can be affected but prevalence increases dramatically with age with a greater incidence in subjects between 40 and 50 years old.[2]

The joints involved are characterized by a breakdown and loss of articular cartilage that leads to a decrease in the joint space and friction between the bones causing swelling, chronic pain, functional impairment, deformity and disability.[3–6]

To date, the hypoxia inducible factor-1 alpha (HIF-1α) has increased its relevance in the assessment of cartilage since its participation is essential in the homeostasis of this tissue.[7] Articular cartilage is a hypoxic tissue in which HIF-1α is of pivotal importance for survival and growth of chondrocytes during cartilage development as well as energy generation and matrix synthesis of chondrocytes in both healthy and pathological conditions.[8,9] By using microarrays, it was also shown that HIF-1α is expressed in human fetal chondrocytes, which means that this transcription factor is essential for development and maintenance of cartilage.[10]

The viability of chondrocytes is compromised by several phenomena such as oxidative stress, inflammatory mediators, biochemical injury and hypoxic conditions. The avascularity of cartilage tissue has allowed establishing well conserved mechanisms where the chondrocytes can survive under such conditions. It is note, that under healthy conditions, oxygen concentration in articular cartilage varies from 0.5 to 10% (~4–70 mm Hg, respectively).[11,12] When oxygen concentration decreases and environment turns increasingly hypoxic, HIF-1α plays a critical role to maintain homeostasis, through induction the expression of a variety of genes encoding proteins to increase the availability of oxygen and nutrients to homeostatic levels.[8,13]

Taking into account of these pieces of information and the recent growing interest in HIF-1α in rheumatic diseases,[14,15] we focused the present review on the potential role of HIF-1α in OA, especially in as some genetic factors may influence in the maintenance or breakdown of articular cartilage.

Methods

Literature review criteria and search strategy

All relevant literature in the field of HIF-1α and OA, published in the last 15 years was reviewed. The search included original articles concerning humans and/or animal models published between January 2000 and December 2015. To identify all available studies, a detailed search pertaining to HIF-1α and OA was conducted according to PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines.[16] A systematic search was performed in the electronic database (PubMed) using the following Mesh search terms in all possible combinations: "HIF-1α" or "osteoarthritis" or "articular cartilage" or "HIF-1α polymorphisms" or "HIF-1α signaling pathway" or "hypoxia" or "rheumatic diseases", and the combined phrases in order to obtain all genetic studies on the relationship of genetic polymorphisms of HIF-1α signaling pathway associated with OA. In addition, the reference lists of all retrieved articles were manually reviewed. Two independent authors (JFT and GAMN) analyzed each article and performed the data extraction independently. Discrepancies were resolved by consensus.

Inclusion and exclusion criteria

We excluded from this review the following types of publications: articles not published in English, case reports, clinical trials, and letters to the editor that were purely commentary. Search results were screened to avoid duplicates. Titles, abstracts, and full reports of articles identified were systematically screened with regard to inclusion and exclusion criteria.

Results

To date, it has compiled important information about the role of HIF-1α in rheumatic diseases. Approximately 5599 publications were identified in PubMed database between January 2000 and December 2015. The results of the search strategy are illustrated in Fig. 1.

General concepts of OA genesis and genetics

In the last years, the knowledge of OA has grown exponentially; however, there are still gaps that have not been possible to address. Currently, cases of OA in very young people are most frequently reported, which gradually induces to change the concept that OA is a disease of elders only. Also, there are very heterogeneous intermediate phenotypes defining the different degrees of severity of OA, since slight crackles of joint, until total loss of articular cartilage. This process is complex, but it is thought that the interaction of biomechanical stress, proinflammatory cytokines, metabolic, environmental, and mainly genetic factors, are the orchestrators that promote the disruption of cartilage homeostasis and initiation of the catabolic pathway.[6,17–22]

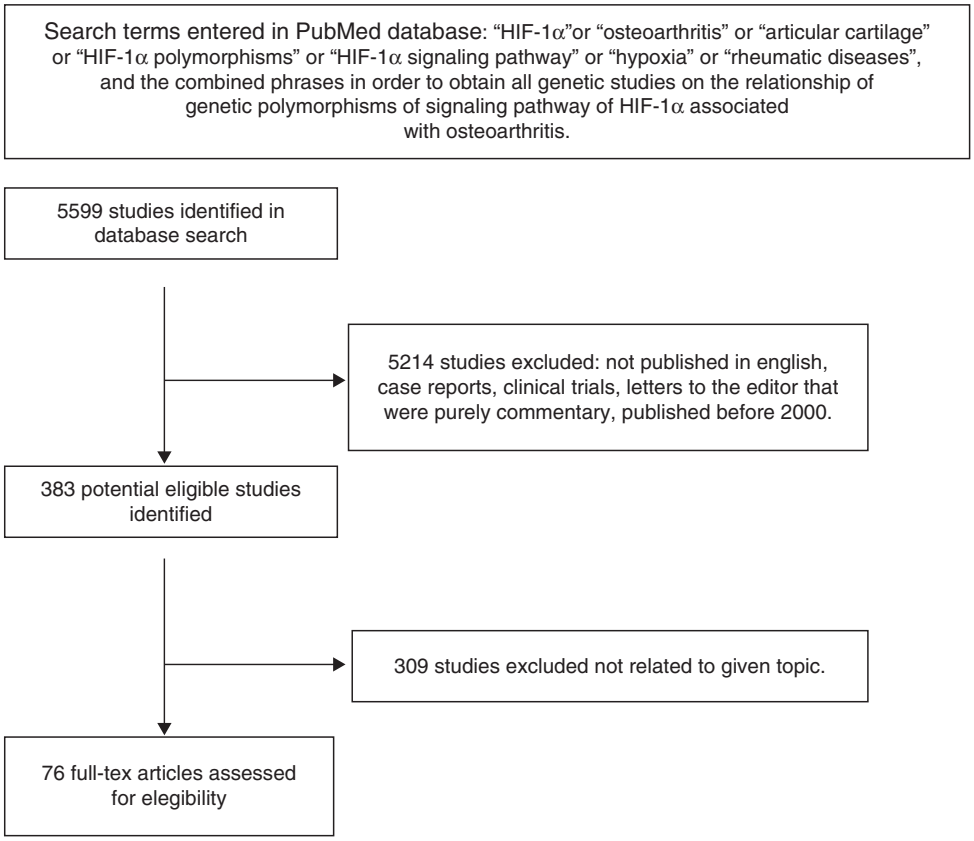

Fig. 1 – Representation of the search strategy.

Although the pathophysiology of OA is not fully characterized, several candidate genes have been reported to be associated with OA susceptibility. Fernández-Moreno et al. determined that despite the multifactorial nature of OA, it not follows the Mendelian inheritance patterns, most likely by the alterations of gene interactions.[20] They analyzed different genes located on different chromosomes, and the results revealed the complexity of this field. Table 1 shows some genes and their relationship with the different phenotypes of OA described in this study.

Meulenbelt published a study aimed to determine which signaling pathways were most important to the development of OA.[6] The most common pathways or genes were the 7q22 *locus* containing multiple potential genes, the growth differentiation factor5 (*GDF5*) gene, frizzled related protein (*FRZB*) gene, the deiodinase iodothyronine, type II (*DIO2*) gene and the *SMAD3* gene.

The genetic bases in OA can further refine the understanding of the genotype–phenotype relationship, through the presence of single nucleotide polymorphisms (SNP). A genetic polymorphism can be a pivot between a mechanism of resistance or susceptibility in a disease. Genetic polymorphisms that affecting a coding or regulatory sequence and produce major changes in protein structure or mechanism of regulation of expression, can result in different phenotypes.[23,24] In Table 2 we show some SNPs with phenotypes well established.

Function and structure of articular cartilage

Articular cartilage is a highly specialized tissue of joints; its principal function is to provide a smooth, lubricated surface for articulation and to facilitate the transmission of loads with a low frictional coefficient. Injury to articular cartilage is recognized as a cause of significant musculoskeletal morbidity. The unique and complex structure of articular cartilage makes treatment, and repair or restoration of its defects challenging for the patient, the surgeon, and the physical therapist. The preservation of articular cartilage is highly dependent on maintaining its organized architecture.[40]

Articular cartilage is the primary target tissue in the degenerative process; there are very particular characteristics that make it different from the others, protruding their lack of capillary network. Articular cartilage consists of extracellular matrix (ECM), proteoglycans, chondrocyte, collagen and water; receives its nutrients and oxygen supply by diffusion from the dynamic flow of synovial fluid and subchondral bone. The regulation of metabolism of articular cartilage involves a vast network of signaling pathways that, in the case of OA, the delicate balance between synthesis and degradation of ECM, is strongly affected. Thus, the osteoarthritic process begins with a decreased resistance to extrinsic stress of chondrocytes, along with changes in the activity of proliferation, energy metabolism and response to growth factors.[41–46] The breakdown of cartilage during the OA pathogenesis is not

Table 1 – Genes associated with the development of OA.

Gene	Chromosome	Phenotypic manifestation
COL2A1, COL11A1, COL11A2	1, 6, 12	Early onset OA
COL9A1	6	Early onset of knee OA
MATN3	2	Early onset of hand and knee OA
COMP	19	Early onset of hip OA hip
COL1A1	17	Reduction of hip OA in women
BMP2	20	Reduction of knee OA in women
TGFB1	19	OA
FRZB, IL4R	2, 16	Hip OA in women
IL1, ASPN, TIMP3	2, 9, 22	Hip and knee OA
IL6	7	Hip OA
AGC1	15	Hand OA
VDR	12	Arthritis in several joints
ERA	6	OA in women
ADAM12, LRCH1, TNA	3, 10, 13	Knee OA
CILP	15	Knee OA in men
CALM1	14	Hip OA in Japanese population
IGF-1	12	Increased risk of OA
IL17	6	Susceptibility to develop OA

Taken and modified from Fernández-Moreno et al.[20]

only related to the loss of ECM but also chondrocytes death. Chondrocyte death by apoptosis, necrosis, chondroptosis, or combination of these processes has been implicated in the pathogenesis of OA.[47]

The HIF-1α system

HIF-1α is a transcriptional factor encoded by the *HIF1A* gene located within chromosome 14q21-24 and is formed by 15 exons; HIF-1α consists of 826 amino acids and it has a molecular weight of 120 kDa.[48] HIF-1α is a heterodimer of two chains, alpha chain (regulated by oxygen) and beta chain, both arranged in a double helix (basic helix–loop–helix, bHLH). There are two nuclear localization signal (NLS), but only that found in the C-terminal position it is responsible for the accumulation of HIF-1α in the nucleus. In the N-terminal region, is located the bHLH and PER-ARNT-SIM A (PAS A) domains, necessary for dimerization and DNA binding through hypoxia response elements (HRE). Finally, the active site of this protein is an oxygen dependent degradation domain (ODDD) that functions as an oxygen sensor (Fig. 2).[49–51]

Under normoxia and in the presence of Fe^{2+} and 2-oxoglutarate, the specific proline residues 402 and 564 are hydroxylated on ODDD domain by prolyl-hydroxylases (PHDs) oxygen dependents, to form a complex with the factor of von Hippel-Lindau (VHL); in turn, this complex binds to ubiquitin (Ub) and it subsequently degraded in the proteasome (Fig. 3).

An external stimulus cellular, as a growth factor that binds to its receptor tyrosine kinase, triggers a cascade of signaling pathways within the cell. For example, vascular endothelial growth factor (VEGF) activates the phosphatidylinositol-3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways (ERK1 and ERK2).[52] PI3K activates the serine/threonine kinase (AKT), and AKT activates the FKBP12 rapamycin-associated protein, mTOR, RAFT (FRAP), which induces the expression of HIF-1α.

Under conditions of reduced oxygen (hypoxia), PHDs activity decreases, which stabilizes HIF-1α and accumulates in the cytoplasm to be phosphorylated by MAPK.[53–55] Once phosphorylated, HIF-1α it translocates to the nucleus and binds to HIF-1β subunit (also known as aryl hydrocarbon nuclear translocator, ARNT) to form the [HIF-1α/HIF-1β] complex. This complex through the HRE, binds to specific DNA sequences 5′TAGCGTGH3′ present in promoter regions of genes for subsequent expression.[47,55,56]

Some of these target genes include nitric oxide synthase 2 (*NOS2*), vascular endothelial growth factor (*VEGF*), erythropoietin (*EPO*), some glucose transporters (*GLUT1, GLUT3*), Insulin-like growth factor type 2 (*IGF2*), which potentially acts in order to maintain the chondroprotective functions challenged by the detrimental conditions occurring in the OA

Table 2 – Single nucleotide polymorphisms (SNP) associated with OA processes.

Gene/dbSNP rs Id	Phenotype	First author [Reference]
WISP1 rs2929970	Susceptibility to spinal OA in woman	Urano et al.[25]
RAGE rs2070600	Susceptibility to knee OA	Han et al.[26]
DVWA rs7639618	Susceptibility to knee OA	Zhang et al.[27]
ACE rs4343, rs4362	Susceptibility to knee OA	Qing et al.[28]
MATN3 rs8176070	Susceptibility to OA	Gu et al.[29]
DIO2 rs225014	Susceptibility to OA	Meulenbelt et al. [30]
ADAMTS14 rs4747096	knee OA in woman (Thai population)	Poonpet et al.[31]
ADAM12 rs1871054	Increased risk of OA	Wang et al.[32]; Kerna et al.[33]
HIF1A rs11549465	Protective role in the loss of articular cartilage	Fernández et al.[34]
IL6 rs1800796	Protective factor for hip and knee OA in the elderly	Fernandes et al.[35]
IL16 rs11556218, rs4072111, rs4778889	Decreased knee OA risk	Luo et al.[36]; Liu et al.[37]
GDF5 rs143383	Protective factor for knee OA	Pan et al.[38]; Tawonsawatruk et al.[39]

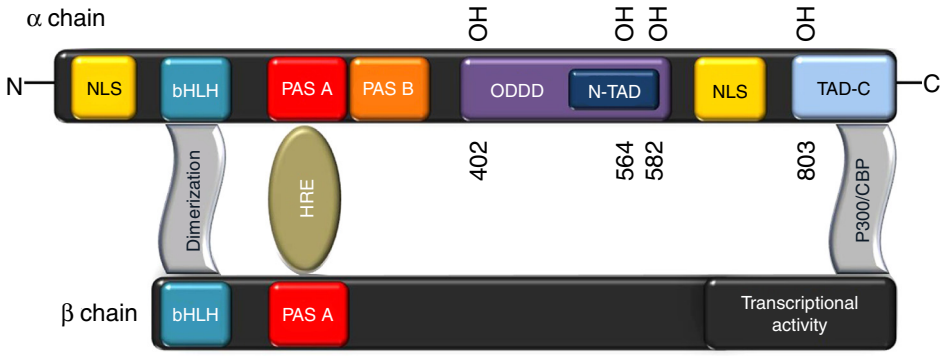

Fig. 2 – Structure of hypoxia inducible factor 1-α (HIF-1α). The NH2-terminal of HIF-1α and HIF-1β consists of bHLH (basic helix–loop–helix) and PAS (Per-ARNT-Sim homology) domains that are required for heterodimerization and DNA binding. The COOH-terminal of HIF-1α (residues 531–826) contains two transactivation domains (TADs). The short half-life of HIF-1α under nonhypoxic and posthypoxic conditions is due to rapid ubiquitination and proteasomal degradation. HIF-1α residues 400–600, this region was designated the oxygen-dependent degradation domain (ODDD).

joint environment (Fig. 4).[8,54–59] This relationship among different genes renders the close relation of HIF-1α with several pathologies.[60–64]

Genetic polymorphisms in the HIF-1α system and their importance in OA

To try to explain simply the interaction of genetic polymorphisms associated with HIF-1α with importance in OA, we divided this system into three stages: (1) genes that activate HIF-1α; (2) proteins that directly interact with HIF-1α; and (3) genes driven by HIF-1α.

Genes that activate HIF-1α system

HIF-1α activation can start by the binding of different proteins to their receptors on the cell membrane; these proteins may be enzymes, growth factors, interleukins or other type molecules; that they can be affected by the presence of genetic polymorphisms which may be associated with the development of OA. Yang et al. analyzed the effect of receptor advanced glycation end products (RAGE) polymorphisms on susceptibility to

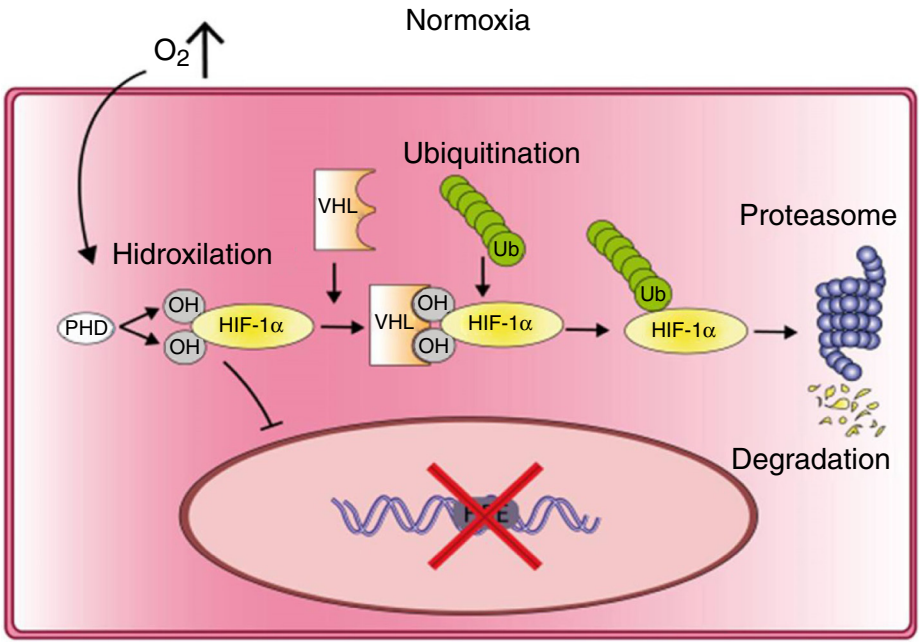

Fig. 3 – HIF-1α activity under normoxic conditions. Under normoxia conditions, the specific proline residues 402 and 564 on ODDD domain are hydroxylated by oxygen dependent prolyl-hydroxylases (PHDs) that leads to the formation of a complex with the von Hippel-Lindau (VHL) factor; which in turn, binds to ubiquitin (Ub) and is subsequently degraded by the proteasome.

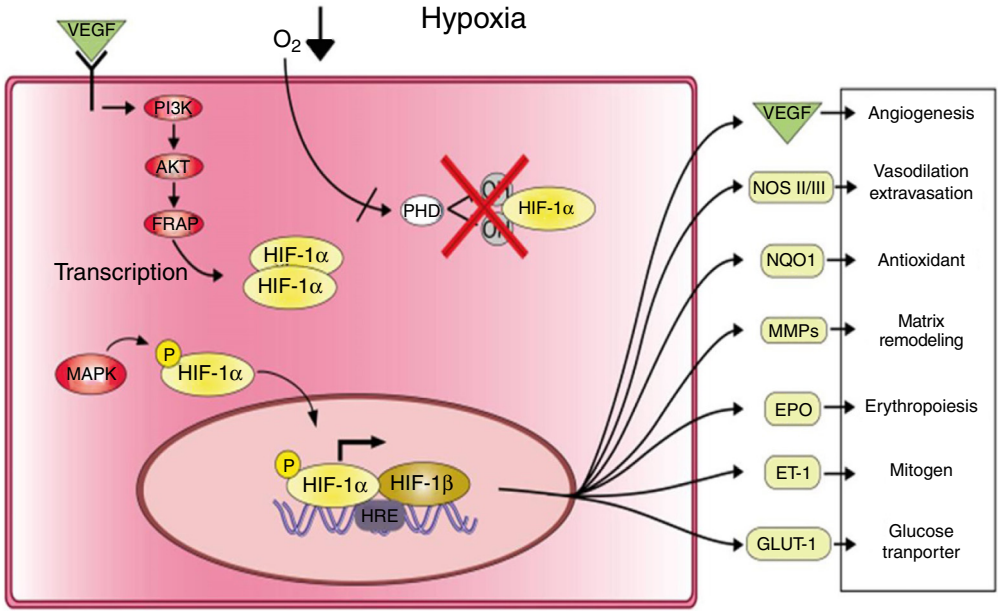

Fig. 4 – HIF-1α activity under hypoxic conditions. Under hypoxic conditions, HIF-1α is stabilized and phosphorylated by MAPK; once phosphorylated, HIF-1α translocates to the nucleus and binds to HIF-1β subunit to form the [HIF-1α/HIF-1β] complex. This complex, through the HRE, binds to specific DNA sequence 5′TAGCGTGH3′ present in the promoter regions of several genes for their subsequent activation.

and severity of OA in a Han Chinese population. *RAGE* participates in regulating inflammation, even in the production of matrix metalloproteinases (MMPs). MMP-1 degrades cartilage, which may result in OA development. They found that two polymorphisms (*rs1800625* and *rs2070600*) in *RAGE* gene showed a significant association between OA patients and healthy controls (OR = 0.42, $p = 0.016$, and OR = 2.78, $p = 0.047$, respectively).[65] In the study performed by Han et al.,[26] they evaluated that the presence of *rs2070600* polymorphism in *RAGE* gene in interaction with obesity, may determine the susceptibility of knee OA.

Swellam et al. reported a potential influence of intereukin-1 receptor antagonist (IL-1RA) gene polymorphism on knee OA risk. *IL-1* gene is supposed to be involved in the cartilage destruction process. In this regard, interleukin-1 receptor antagonist (IL-1RA) competing with IL-1 for binding to its receptor may act as an inhibitor of cartilage breakdown. They conducted a case–control study with knee OA patients, and concluded that IL-1RN*2 allele represent a significant factor influencing the severity and course of knee OA ($p = 0.002$).[66]

Fernandes et al., analyzed the influence of pro-inflammatory cytokine IL-6 with severity and functional status of OA in elderly individuals, and determined that the *rs1800796* polymorphism is a protective factor for the presence and severity of hip and knee OA in the elderly. The individuals harboring the *C* allele have lower prevalence and severity of OA when compared to individuals without this polymorphism.[35]

Meanwhile, interleukin-16 (IL-16), a pleiotropic cytokine, plays a fundamental role in inflammatory diseases. Liu et al. determined that, compared with the *C/C* genotype, the *C/T* genotype increased the risk of primary knee OA in *rs4072111* of *IL-16* gene (OR = 1.83); however, compared with the *T/T* genotype, the *T/G* genotype decreased the risk of primary knee OA in *rs11556218* polymorphism (OR = 0.37).[37] Likewise, Luo et al. evaluated the same polymorphisms and determined the same behavior.[36] These results suggest that *IL-16* gene polymorphisms are associated with the risk of knee OA.

Finally, there are other genes associated with activation of HIF-1α, such as *PIK3R1*, *AKT2*, *GSK3B*, *IL6*, and it will be necessary to explore their genetic variants and to determine their participation in the development of OA.

Proteins that interact directly with HIF-1α

The stabilization of HIF-1α in the cytoplasm basically depends on the hydroxylation at specific sites within of the ODDD domain. However, the presence of genetic polymorphisms could alter the structural properties of the transcripts, and this may influence the susceptibility or resistance to diseases, such as was analyzed by Uchanzka et al. in autoimmune diseases associated with HLA-B*27 allele.[67]

In 2003 Tanimoto et al. demonstrated that the substitution of proline by serine in the 582 (P582S) position, due to the presence of single nucleotide polymorphism (*rs11549465*) within *HIF1A* gene, enhances its transcriptional activity,[56] due to an alteration in the characteristics and properties of the binding sites with the target genes.[67,68] Recently, our group evaluated the presence of this polymorphism in samples of patients with OA, finding that was associated positively as a protective factor in cartilage loss (*CT* genotype OR = 0.2, $p = 0.003$,

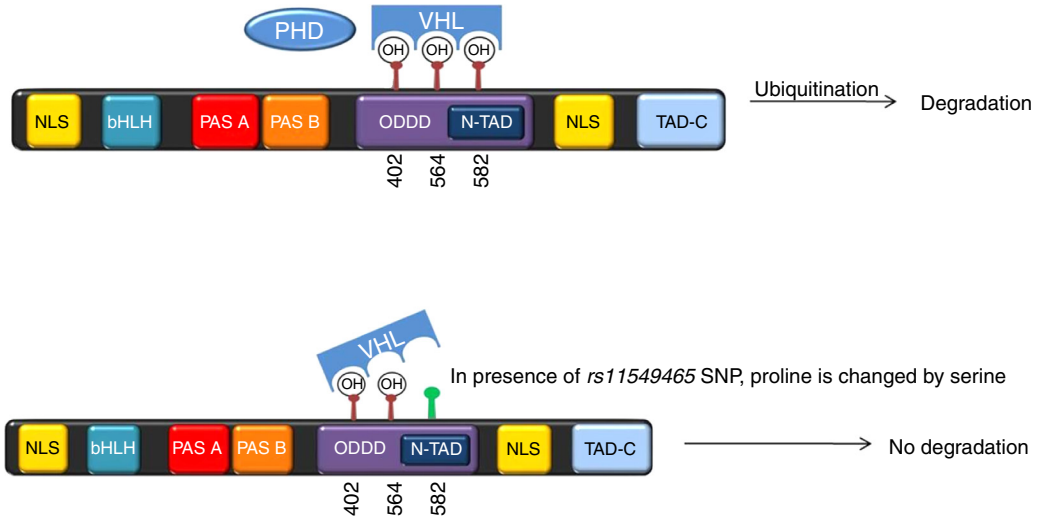

Fig. 5 – The presence of *rs11549465* polymorphism, generates a change of proline by serine, which confers greater stability to the HIF-1α protein due to poor interaction between VHL and hydroxylation sites within the ODDD.

or *T* allele OR = 0.2, $p = 0.004$).[34] This phenomenon may be explained by the fact that the presence of this polymorphism confers greater stability to the HIF-1α protein due to poor interaction between VHL and hydroxylation sites within the ODDD (Fig. 5).

Other polymorphism medically important, with increased transcriptional activity within *HIF1A* gene that also was tested by Tanimoto et al., is Ala588Thr (*rs11549467*). Similarly, we evaluate this polymorphism in patients with OA, but did not find him associated. However, this opens the possibility to be evaluated in other populations in order to better understand their influence in OA.

Additionally, it have been described polymorphisms for *EGLN1* (also known as PHD2, prolyl-hydroxylase 2), *VHL* and *HIF1AN* (*HIF1A* inhibitor factor) genes interacting directly with HIF-1α, but not have been evaluated in OA and that may be of clinical importance in this disease. Some studies in rheumatoid arthritis (RA) demonstrated the expression and regulation of prolyl hydroxylase domain (PHD) enzymes and factor-inhibiting HIF-1α (FIH-1), which regulate cellular HIF-1α levels. It is known that RA is characterized by hypoxia and the expression of hypoxia-inducible transcription factors (HIFs), which coordinate cellular responses to hypoxia. Muz et al. conducted this study in RA fibroblast-like synoviocytes, and concluded that PHD-2 is the major hydroxylase regulating HIF levels and the expression of angiogenic genes in arthritic cells. PHD-2 appears to regulate responses relevant to arthritis via HIF-α, highlighting the major importance of this enzyme in hypoxia- and angiogenesis-dependent inflammatory diseases.[69] This makes us suppose that the presence of genetic polymorphisms of these genes may affect the stability of HIF-1α contributing importantly in the OA disease.

Genes driving by HIF-1α

OA chondrocytes are metabolically active, displaying increased synthesis of type II collagen. In comparison with healthy cartilage, OA articular chondrocytes exhibit increased *in vivo* synthesis of collagen prolyl-4-hydroxylase type II, a pivotal enzyme in collagen triple helix formation.[70] Once stabilized HIF-1α in the cytoplasm, several downstream genes are expressed in order to restore multiple components of the extracellular matrix. The presence of genetic polymorphisms in these genes may alter the function of specific proteins that restore joint tissues, promoting the development of OA.

Raine et al. performed an allelic expression analysis of the OA susceptibility gene *COL11A1* in human joint tissues. By using RNA from OA cartilage of individuals undergoing elective joint replacement for OA of the hip (total hip replacement, THR) or of the knee (total knee replacement, TKR), they observed a significant allelic expression imbalance (AEI) at *rs1676486* ($p < 0.0001$) with the *T*-allele correlating with reduced *COL11A1* expression. AEI at *rs1676486* is a risk factor for lumbar disk herniation, but not for OA.[71]

Rodríguez-Fontenla et al. conducted a meta-analysis of nine GWAS to assess candidate genes for association with OA, and only 2 of the 199 candidate genes (*COL11A1* and *VEGF*) were associated with OA in the meta-analysis. Two polymorphisms in *COL11A1* gene (*rs4907986* and *rs1241164*) showed association with hip OA in the combined analysis (OR = 1.12, $p = 1.29 \times 10^{-5}$, and OR = 0.82, $p = 1.47 \times 10^{-5}$, respectively); and the *rs4908291* with the sex stratified analysis in women only (OR = 0.87, $p = 1.29 \times 10^{-5}$). Other polymorphism in *VEGF* gene (*rs833058*) showed association with hip OA in men only (OR = 0.85, $p = 1.35 \times 10^{-5}$).[72]

The oxygen and nutrients supply to articular cartilage is by diffusion from the synovial fluid. The role of vascular endothelium growth factor (VEGF) is critical for angiogenesis in subchondral bone.[8] There are only few studies related with gene variants of *VEGF* gene that may contribute to the development and progress of OA.

Sánchez et al. evaluated two polymorphisms of *VEGF* gene, −460T/C and +405C/G, in patients with knee OA and compared

with healthy controls, but did not find association.[73] Yuan et al. conducted a meta-analysis to order understand the relationship between the pathogenesis of OA and the expression levels of VEGF in multiple disease tissues in these patients. A total of 11 case–control studies, containing 302 OA patients and 195 healthy controls, demonstrate that VEGF expression levels in OA patients are significantly higher than healthy controls (standardized mean difference = 1.18, 95% CI: 4.91–9.11, $p < 0.001$), and these levels strongly correlate with the pathogenesis of osteoarthritis.[74]

One of the mechanisms of cartilage degradation in OA is enzymatic proteolysis of the extracellular matrix by metalloproteinases. MMP-1, produced by chondrocytes and synovial cells, is a major protease of the MMPs family.

Barlas et al. evaluated three polymorphisms in the promoter of matrix metalloproteinase-1 (*MMP-1*), *MMP-2* and *MMP-9* genes in patients with knee OA and compared with ethnically matched control. They found significant differences between the groups regarding the genotype distribution of *MMP-1* polymorphism ($p = 0.001$). The frequencies of 1*G*/1*G* and 1*G*/2*G* genotypes were significantly higher in the knee OA than in the controls ($p = 0.002$, and $p = 0.006$, respectively). In addition, 1*G* allele frequency of *MMP-1* gene was higher in the patients than in the control group ($p = 0.0001$). The genotype distributions and allele frequencies of *MMP-2* and *MMP-9* gene polymorphisms did not differ between the OA and the control groups ($p > 0.05$). These findings suggest that the −1607 1*G*/2*G* polymorphism (*rs1799750*) in the *MMP-1* gene may contribute to susceptibility to knee OA.[75]

Similarly, Lepestos et al. evaluated the *rs1799750* polymorphism in *MMP-1* gene, but they did not find significant association in crude analysis; however, after multiple logistic regression analysis, 1*G*/2*G* was associated with reduced odds of knee OA by 75% in males, compared to genotypes 1*G*/1*G* + 2*G*/2*G*, adjusting for age and BMI (adjusted OR = 0.25, $p = 0.035$).[76]

Finally, Honsawek et al. analyzed the *MMP-3* (*rs3025058*, −1612) polymorphism with knee OA patients. The 5*A* allele frequency was indicated as 15.5%, and 6*A* allele was as 84.5% in OA patients, whereas it was 10–90% in the control group. Accordingly, the present study has indicated that the −1612 5*A*/6*A* polymorphism genotypes of *MMP-3* gene promoter do not play a role in the development of OA.[77]

These results suggest that the MMPs family activity is influenced by presence of genetic variants, which would break the balance between synthesis and degradation of extracellular matrix, and this condition may contribute to susceptibility of OA.

Nitric oxide (NO) is essential in the maintenance of vascular tonus and the presence of endothelial impairment (reduced vascular relaxation) may suggest a problem regarding the NO pathway. NO is produced by endothelial NO synthase (eNOS), and its production can be influenced by polymorphisms of the *eNOS* gene.[78] To date, no studies have been done related with OA and genetic polymorphisms of NOS; however, several genetic polymorphisms in the *eNOS* gene are associated with the pathogenesis of RA.

The level of NO is increased in RA patients, and a study suggested that NO can regulate the balance of Th1/Th2 in autoimmune diseases, and it was a key mediator of apoptosis within rheumatoid arthritis joints. An et al. studied two polymorphisms of the *eNOS* gene (*rs2070244*, T-786C; and *rs1799983*, G894T) in patients with RA, and observed that individuals with the −786*CC* genotype have an increased risk of RA.[79]

Brenol et al. evaluated the T-786C polymorphism in RA patients comparing with extraarticular manifestations. They found that the *C* allele was significantly associated (p corrected = 0.032), suggesting the participation of the T-786C polymorphism of the *eNOS* gene and RA.[80]

These results we make suppose that *eNOS* gene polymorphisms can have an important impact on development of OA; it will be necessary to explore these genetic variants to corroborate it.

The erythropoietin-mediated bone marrow response to anemia is under the control of hypoxia-inducible factors (HIFs), the master regulators of oxygen and iron homeostasis. The hypoxic characteristics of joint cartilage make that HIF-1α participates actively allowing transcription of target genes. Erythropoietin (*EPO*) gene is expressed after of an increase of HIF-1α on cytoplasm.[81,82] But to date, no scientific evidence that supports the possible association between erythropoietin and cartilage loss in OA; and even more, the presence of polymorphisms in *EPO* gene could represents an important factor associated with risk of OA.

Clinical relevance of PHDs inhibitors as potential therapeutic targets in OA

To date, HIF-1 inhibitors are classified by their HIF inhibitory mechanism, including affecting on HIF-1α protein level, HIF-1 dimerization, HIF-1 DNA binding, or HIF-1α transcription of target genes.[83] Due to the results described above, the main objective for achieving a therapeutic effect in the treatment of OA at cartilage level, could stabilize HIF-1α in the cytoplasm so that it can induce the expression of restoration genes. Naturally, there are genetic polymorphisms that increase the transcriptional activity of HIF-1α in comparison with common isoform. At the experimental level, has evaluated the Dimethyloxallyl Glycine (DMOG), a potent inhibitor of prolyl-hydroxylases.[84] The endogenous HIF-1α levels can be increased by the suppression of PHD activity, either by reducing the cellular oxygen level or by combining the Fe (II) competitively. DMOG is a cell permeable, competitive inhibitor of the PHDs. DMOG is an analog of 2-oxoglutarate, and in this way it inhibits not only the HIF prolyl but also asparaginyl hydroxylases. Beside that it is predicted to inhibit other members of 2-oxoglutarate-dependent dioxygenases. There are three HIF-prolyl hydroxylases known in mammals, and they are encoded by separate genes: *PHD1*, *PHD2*, and *PHD3*. Like all 2-oxoglutarate-dependent dioxygenases, PHDs require oxygen for hydroxylation, as well as tricarboxylic acid cycle intermediate, 2-oxoglutarate (α-ketoglutarate), iron (Fe^{2+}), and ascorbate as cofactors. When oxygen levels are low, HIF-1α escapes PHD hydroxylation and recognition by the VHL.[85,86]

Other inhibitors of PHDs with potential beneficial effects are deferoxamine (DFO) and cobalt chloride ($CoCl_2$), an iron chelator and a competitive inhibitor of iron, respectively; they are routinely used both *in vitro* and *in vivo* to inhibit PHDs activity by competing for endogenous iron (II). Other iron chelators, such as ciclopirox olamine, and competitive inhibitors of iron, such as Cu^{2+}, Zn^{2+}, and Mn^{2+}, are also used as PHDs inhibitors.[53]

Likewise, there are studies showing that HIF-1α and its target gene *VEGF*, are critical regulators of angiogenic–osteogenic coupling.[7,87] In addition to chondrocytes, osteoblasts also express HIF-1α and promote skeletal vascularization during endochondral bone formation; manipulation of the HIF system via pharmacological or genetic approaches, is an attractive strategy for treating hypoxic diseases, including skeletal diseases such as subchondral bone inflammation during OA.[53]

Conclusion

Is worth mentioning that several associations' studies between SNPs and OA disease remain unconfirmed or controversial due to bias in patient enrolling criteria differences in OA affected joint sites, in classification and staging mode.

Cartilage destruction in OA mediated by catabolic enzymes and chondrocyte death, including apoptosis and/or autophagy, also contribute to the pathogenesis. The studies showed that the expression of HIF-1 is increased in OA cartilage to mediate the response of chondrocytes to hypoxia; HIF-1 acts as a survival factor by enhancing extracellular matrix synthesis and inhibiting apoptosis[88]; HIF-1 serves to regulate both autophagy and apoptosis and HIF-1 is of pivotal importance in cartilage homeostasis.[89] Also, it will also be necessary to explore other isoforms of HIF, such as HIF-2α, which it seems to have the opposite effect to HIF-1α. The HIF-2 protein acts as a brake on the autophagy-accelerator function of HIF-1, and promotes chondrocyte hypertrophy, a terminal differentiation state characterized by a unique gene expression program, including type X collagen and the type II collagen-degrading protease MMP-13.[9]

Although further studies should elucidate the exact mechanism of HIF-1 in OA the current evidence induce to consider it as a promising approach to the treatment of OA. However, the results have been reported to indicate that genetic markers could contribute to the understanding of the natural history of this disease.

Conflict of interest

The authors declare no conflicts of interest.

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