

Cartilage Regeneration Induced by HIF-1 α Through Different Pathways

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Abstract. In practical practice, cartilage damage is a prevalent medical issue. Conventional cartilage replacement techniques do not meet the objectives of comfort medicine due to the limitations of invasive surgery, specific longevity, possible rejection, etc. Previous researches have revealed that HIF-1 α is known as a crucial chondrogenic regulatory factor and elevated levels of HIF-1 α seems to have been essential in angiogenic remodelling, erythropoiesis and cell proliferation. This paper reviews the definition of HIF-1 α and the factors that influence it, and elaborates four mechanisms of HIF-1 α -induced cartilage regeneration in the molecular level: modulating transcriptional genes such as VEGF, adapting the cell adhesion pathway of bone marrow mesenchymal stem cells, intervening Protein Serine Kinase signaling pathway and HIF1 α /FAK signaling pathway, which offers new ideas for inducing cartilage regeneration at the molecular level. HIF-1 α -induced neovascularisation provides a rich blood supply to areas of cartilage that are deprived of blood supply and promotes the repair and regeneration of damaged cartilage. Consequently, more research on HIF-1 α activation for cartilage regeneration may provide further perspectives into the treatment of clinical cartilage defects.

Keywords: HIF-1 α , Chondrogenesis, Cartilage Regeneration, Signaling Pathways, Cell Signaling.

1. Introduction

Cartilage with only 2–4 mm thick is a special structure on the surface of the joint bone, which is elastic, smooth and made up of cartilage cells and matrix. There is almost no friction on the cartilage surface. Injuries can cause the cartilage to thin or even break. As cartilage is a tissue that lacks blood supply, poor blood flow in cartilage injuries tend to form adhesions in the surrounding tissues, mostly complicating traumatic chondritis. It is commonly caused by sports injuries or trauma that cartilage is damaged. Due to cartilage's limited capacity for healing, cartilage injuries are difficult to treat. Numerous traditional techniques, such as drilling and microfracture, can be used to treat cartilage injury. The common clinical management is to replace the damaged cartilage with an artificial arthroplasty. However, conventional artificial joint replacement is an invasive procedure with a prognosis of complications such as peri-articular infection, fracture and loosening of the prosthesis. Moreover, the procedure is expensive and different types of prostheses have different lifespans and will need to be replaced at the end of their lives. Recently, a new therapeutic approach to induce cartilage regeneration has attracted a lot of attention from researchers. The use of Hypoxia-inducible factor-1 α to produce cartilage regeneration allows patients to have less pain, smaller incisions, less risk of complications and better joint flexibility. HIF-1 α offers a new approach to comfortable medical treatment for cartilage regeneration. HIF-1 α is a regulatory protein depending on oxygen that monitors the level of oxygen in the surrounding area of the cell. It is influenced by mechanical factors (mechanical stretching、shear stress and matrix stiffness) and biochemical factors (pH and biological agent). The oxygen-dependent degradation domain in HIF-1 α acts as a mediator for oxygen-regulated stability. HIF-1 α is involved in a variety of physiological reactions to hypoxia, including erythropoiesis, glycolysis, and angiogenesis, which provide more lasting relief [1]. Through the microtubules, HIF-1 α can enter the nucleus and form dimers with other HIF-1 α molecules inside. These dimers then attach to the hypoxia reaction element to create binding proteins. Transcription of HIF-1 α target genes is subsequently activated by these initiating transcription factor complexes, stimulating angiogenesis, providing a blood supply to the cartilage region and inducing cartilage

regeneration. This paper explores the activation of HIF-1 α and its key role in cartilage regeneration. In addition, several factors targeting the activation of HIF-1 α are presented. This review focuses on four pathways of HIF-1 α -induced cartilage regeneration: (1) Regeneration of condylar cartilage induced by HIF-1 α by regulating the transcriptional expression of genes, such as VEGF, thus promoting angiogenesis; (2) Cartilage-specific gene expression enhancement enhanced by HIF-1 α through the AKT pathway to induce condylar cartilage regeneration; (3) Repair and regeneration of articular cartilage by HIF-1 α by regulating the cell adhesion pathway of bone marrow mesenchymal stem cells (BMSCs) in vitro; (4) Repair and regeneration of articular cartilage by HIF-1 α through the HIF1 α /FAK signaling pathway. By summarizing the progress of research on HIF-1 α and cartilage regeneration and the potential implication of HIF-1 α in angiogenesis process, the critical induction and repair of cartilage by HIF-1 α is well highlighted as an important mechanism.

2. The role of HIF-1 α and the factors influencing HIF-1 α

HIF-1 α is a crucial controller in charge of activating genes that help cells and the entire organism adapt and survive hypoxic conditions. HIF-1 α belongs to the protein family known as bHLH-PAS. The fundamental region downstream allows for precise interacting with the HRE DNA sequence, and the bHLH and PAS motifs are necessary to synthesize heterodimers with HIF-1 α and HIF-1 β subunits. In the C-terminal half of the HIF-1 α protein, two transactivation (transcriptional stimulation) domains, N-terminal and C-terminal, were later discovered. Oxygen-regulated stability is mediated by the oxygen-dependent degradation domain found in HIF-1 α . HIF-1 α is extensively produced in both mankind and animal tissues that contributes to a variety of biological responses to oxygen deficit, including erythropoiesis and glycolysis, which provide longer lasting relief from oxygen deficiency [1].

The primary conventional factors influencing HIF-1 α production are hypoxia, mechanical stimulation, matrix stiffness, temperature, and pH. Additionally, more and more new stimuli are now known to stimulate the creation of HIF-1 α , including Flavonoid Compound Icarins and 3D bioprinted gradient structures of cartilage scaffolds loaded with BMSCs.

Hypoxia triggers high HIF-1 α expression by activating the HIF-Notch-VEGF signaling pathway. By using Western blot, the three proteins (HIF-1 α , NICD, and VEGF) were similarly expressed, with the hypoxia group exhibiting the greatest expression levels that were considerably greater than normoxic condition group [2]. In circumstances of atmospheric oxygen, induction and stabilization of HIF-1 α can be inspired by low shear stress through NF- κ B and Cezanne-dependent mechanism, which activates endothelial cell for increased HIF-1 α activation in response to hypoxic signaling [3]. High substrate stiffness upregulates the mRNA level of HIF-1 α and low pH(6.0) impairs the mechanism of HIF-1 α degradation outside the nucleus resulting in a rapid increase in HIF-1 α levels [3]. Previous research has demonstrated that HIF-1 can activate the angiogenesis, synovitis, tumor progression pathways [1,2]. Similarly, HIF-1 α is explored as a novel factor to better induce cartilage regeneration.

3. Four mechanisms of HIF-1 α -induced cartilage regeneration

3.1. Regeneration of condylar cartilage induced by HIF-1 α through adjusting the transcriptional genes

HIF-1 α is an oxygen-dependent regulatory protein that senses the oxygen concentration around the cell. It has a very short half-life (<5 min) in a hyperoxic environment, and can be degraded by special proteasomes. When the intracellular oxygen concentration is less than 6%, HIF-1 α remained stable and aggregate. After HIF-1 α transferred to the nucleus, joined HIF-1 β to create dimers and bound to 5'-TACGTG-3' sequence of hypoxia reaction element to form HIF-1 α p300/CBP cyclic adenylate reaction element binding protein. These initiating complexes of transcription factors in turn activated the transcription of HIF-1 α target genes and caused a series of hypoxia adaptations of

histiocytes, such as angiogenesis and remodeling, energy metabolism, erythropoiesis, cell proliferation, and apoptosis [4-6].

Collagen prolyl 4-hydroxylase (C-P4H) is the basis for collagen to maintain a stable helical structure and is a key step in collagen synthesis. HIF-1 α activates the expression of C-P4H and promotes collagen production, thereby maintaining the stability of the extrachondroblast matrix. The amount of glycosaminoglycan (GAG) secreted by chondrocytes at low levels of oxygenation without HIF-1 α is 35% of that of normal oxygen, and the amount of GAG produced by cells at low levels of oxygenation without HIF-1 α is much higher than that of normal oxygen [6]. Thus, it can be seen that HIF-1 α is of great significance for maintaining the proliferative chondrocyte activity and extracellular matrix production. Due to the lack of vascularity in cartilage tissue, it is necessary to induce vascular invasion to deliver large amounts of energy to cartilage tissue in order to accommodate cartilage tissue development and defect repair. By using Western Blot and RT-PCR, HIF-1 α , Notch1 intracellular domain (NICD, an active Notch1 variant), and VEGF were evaluated. HIF-1 α , Notch, and VEGF expression levels were considerably higher in the ischemia group than in the non-ischemic group, showing that ischemia stimulated the HIF-1 α -Notch-VEGF signaling cascade [2]. When cartilage damage lead to tissue edema and hypoxia, HIF was induced to be produced, promoting the expression of VEGF by mast layer chondrocytes and inducing matrix metalloproteinases to degrade the extracellular matrix, thus participating in joint remodelling, which upregulated VEGF to very high multiples within 1 min [6]. This indicated that HIF-1 α may promote endochondral angiogenesis by upregulating the transcriptional expression of genes such as VEGF, thereby regulating endochondral ossification and cartilage modification.

Regarding experimental research, microvessel density (MVD) was strongly positively correlated with the chondromalacia score, HIF-1 α , NICD, VEGF score, and their mRNA expression in the Spearman's correlation coefficient (Tables 1 and 2), and the positive expression was focused on vascular endothelial cells, indicating that angiogenesis is closely related to HIF-1 α , VEGF, and NICD [2].

Table 1. Association of MVD with chondromalacia score, HIF-1 α , NICD, VEGF score in Spearman's correlation coefficient

SD Group	Chondropathy Scores	MVD	HIF-1	Notch-1
MVD	0.788			
HIF-1	0.850	0.626		
Notch-1	0.785	0.576	0.758	
VEGF	0.661	0.627	0.508	0.636

Table 2. Relation between the mRNA expression levels of all groups' linked components

	HIF-1 α	Notch-1	VEGF
Notch-1	0.772		
VEGF	0.810	0.818	
Hes1	0.870	0.879	0.866

3.2. Regeneration of condylar cartilage by HIF-1 α through the protein-serine-threonine kinase (AKT) pathway

It has been demonstrated that the expression of inflammatory factors, typically interleukin 1 and tumor necrosis factor, was abnormally high in the articular cartilage of osteoarthritis patients, stimulating the expression of inflammatory factors or enzymes, such as MMP-13 and prostaglandin E2, which severely damaged cartilage and cartilage matrix. These inflammatory factors can either directly or indirectly stimulate the enhanced transcriptional activity of HIF-1 α [7]. Through the AKT pathway, HIF-1 α activation increases cartilage-specific genes like SOX-9. Whenever HIF-1 α is temporarily deactivated, SOX-9, the principal downstream domains of a transcription factor of cartilage formation, the genes encoding collagen type II and aggrecan, as well as major components

of cartilage tissue, all exhibit decreased expression. Additionally, ischemia and the HIF-1 α pathway have been discovered to control the creation of this matrix, while in normal cartilage this matrix can be predominated by collagen type II and proteoglycan blend. The activity of HIF-1 α controls the production of extracellular matrix (ECM), and stability of HIF-1 α encourages the development of ECM specific to cartilage via increasing SOX9 and altering collagen type II post-translationally [4,6,8,9]. Additionally, HIF-1 α also decreases reactive oxygen species generation, inhibits c-Jun N-terminal kinase activation, and increases the expression of X-linked inhibitor of apoptosis protein-2 (XIAP-2) and B-cell (Bcl-2). Inhibiting chondrocyte apoptosis and promoting chondrocyte survival is achieved by various routes, including levels of anti-apoptotic protein expression. The nuclear factor-B (NF-B) activation process can be inhibited by estrogen receptor (ER) via the HIF-1 pathway, decreasing the inflammatory response [6].

In terms of experimental study, MSCs were treated with LY294002, an AKT inhibitor, before being exposed to hypoxia. HIF-1 α moves to the nucleus during hypoxia, but when LY294002 is present, the hypoxic stimulation does not cause HIF-1 α to accumulate in the nucleus. This finding indicates that hypoxia causes Akt to become phosphorylated and that Akt is an upstream signaling molecule involved in the stabilization and HIF-1 α nuclear displacement brought on by hypoxia [4].

3.3. Regeneration of articular cartilage by HIF-1 α through regulating the cell adhesion pathway of BMSCs in vitro

Articular cartilage damage, the most common musculoskeletal condition, is associated with injury of joint, and because it significantly worsens joint impairment, it affects patients' everyday activities and their life satisfaction. The limited self-regeneration capacity of articular cartilage makes cartilaginous regeneration a challenging process. Medically accessible joint replacements experience issues such aseptic loosening or infection and are unable to rebuild with host joint tissue properly. The greatest solution to these issues, according to recent research, is biological regrowth of the cartilage tissue. The fact that the bone-cartilage compound tissue, which is composed of cartilago articularis and subchondral bone joined, guarantees the joint's regular function and highlights the importance of concomitant healing of skeletal compound tissue for effective knee surgery. A biomimetic scaffold for joint reconstruction using 3D bioprinting that is anisotropic gradient-structured was created [10]. Transcriptome sequencing confirmed the detection of pore size-dependent regeneration of cartilage and mesenchondrium production in a 3D bioprinted cartilage scaffold.

Past studies have shown that 3D bioprinting cartilaginous structures with tiny holes in the top layer can provide adequate mechanical characteristics and a stronger extracellular matrix to produce exterior fibrocartilage, while constructs with large holes in the innermost region can maximize nutrient infiltration via greater angiogenesis as well as provide greater oxygen pressure, in order for the cartilaginous structures can be more rigid [10-13].

Furthermore, through regulating the cell adhesion pathway of BMSCs in vitro, the pore size-dependent cartilage formation was mediated by HIF-1 α .

HIF-1 α may play a role in small pore size (SPZ)-modulated chondrogenesis through controlling the focal cell adhesion signaling pathway, according to microarray profiles [10]. These results showed that HIF-1 α modulated BMSCs' in vitro focused cell adhesion signaling, which in turn aided chondrogenesis and avoided chondrogenic hypertrophy. Sox9 and COL2 α 1 are key transcription factors of chondrocyte differentiation. HIF-1 α upregulation considerably improved chondrogenesis with elevated expression of Sox9 and COL2 α 1 and reduced levels of hypertrophic markers COL10A1 and RUNX2. The latest research confirms, increased cartilage marker genes mRNA induced by Flavonoid Compound Icaritin involving Sox9, Col2 α 1, Aggrecan and the gene Col10 α 1, fostering chondrocyte differentiation, growth, and production of the extracellular matrix has been shown to be related to increased expression and activation of HIF-1 α in chondrocytes [14]. The pre-treated BMSCs with changed HIF-1 α expression were seeded in LPZ and SPZ 3D-printed scaffolds to confirm the HIF1 α /FAK axis in SPZ-regulated chondrogenesis. HIF-1 α downregulation reduced the HIF1 α /FAK

signal and hindered the chondrogenic properties produced by the SPZ scaffold, whereas chondrogenesis through the HIF1 α /FAK signal axis was considerably recovered in LPZ scaffolds with BMSCs [10]. These findings demonstrated that the HIF-1 α regulated cell adhesion signaling pathway affected the SPZ scaffold's BMSCs' pore size-dependent chondrogenesis. In terms of experimental study, Icariin (10⁻⁶ M) was applied to the alginate-chondrocyte 3D cultures in the 3D culturing system for algae and chondrocytes for 21 days. By using real-time PCR, Sox 9, Aggrecan, Col2 α 1 and Col10 α 1 mRNA was measured when compared to the untreated comparison group's results (Figure 1) [14]. This finding implied that Sox9, Col2 α 1, Aggrecan and the gene Col10 α 1 were upregulated via Icariin to promote HIF-1 α activation by chondrocytes and stimulate chondrogenesis in alginate and chondrocyte 3D cultures.

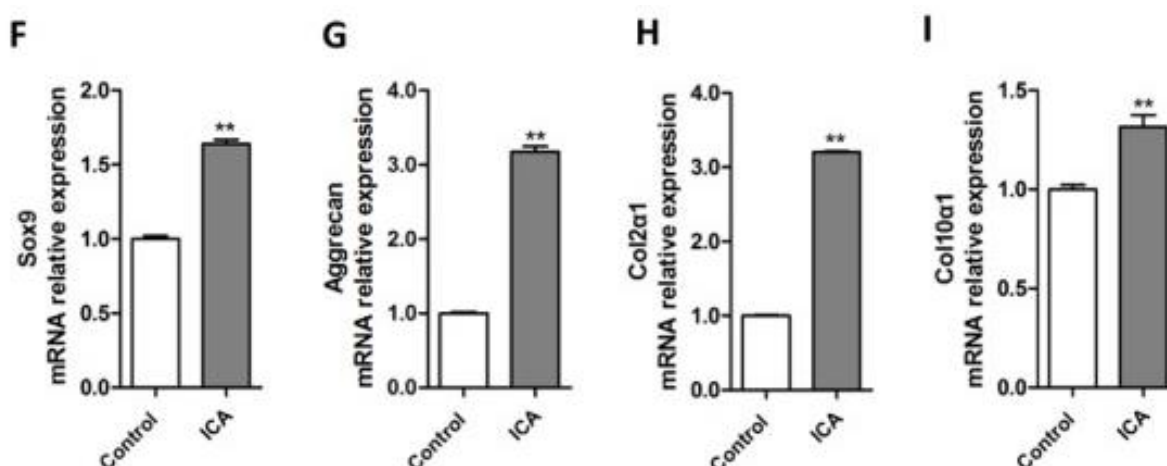


Figure 1. Quantitative analysis of Sox 9, Aggrecan, Col2 α 1 and Col10 α 1 mRNA expression levels [14].

3.4. Repair and regeneration of articular cartilage by HIF-1 α through the HIF1 α /FAK signaling cascade

The functional cartilago articularis regeneration was explored by employing a model of full-thickness cartilage damage in the rabbit knee and the underlying signaling cascades.

Study shows that the gradient structural scaffolds controlled by the HIF1 α /FAK signaling pathway can enhance microvascular development and trigger region-specific cartilage gene as well as ossification indicator [10,15-17]. Significantly, the function of the gradient scaffolds was significantly better than that of non-gradient scaffolds. Besides, histomorphological research demonstrates [10], the Gradient group substantially regenerated hyaline-like cartilage and cell morphology that was more similar to natural cartilage and was more flexible and physiological than artificial joints. These outcomes indicate that in vivo, the pore size-dependent anisotropic cartilage was more effectively repaired and joint was better protected by using a gradient-structured scaffold created using 3D bioprinting. Recent research has also demonstrated that HIF-1 α , which inhibits NF- κ B signaling pathway, plays an anti katabolic role that preserves cartilago articularis [18]. By increasing the HIF1 α /FAK signaling pathway and suppressing NF- κ B signaling pathway, HIF-1 α can promote cartilage regeneration and preserve cartilage function.

In terms of experimental study, specific areas of the gradient structural scaffold for HIF1/FAK in vivo were subjected to immunofluorescence staining tests using HIF1 in red and FAK in green. Comparing the gradient group to the NG750 and NG150 groups, immunostaining revealed that HIF1 and FAK were expressed more strongly within the gradient group's topmost layer [10].

4. Conclusions

Cartilage damage is the key to preventing people from moving and supporting their joints. The current clinical treatments are mostly invasive and can cause unnecessary pain to patients. An oxygen

deficiency transcriptional factor is HIF-1 α . Under the influence of many stimulating factors, stable HIF-1 α assembles and through nuclear pore transfers to the nucleus from the cytoplasm., where it starts transcription, thus inducing cartilage regeneration. Compared with traditional treatment, further investigation into the new treatment strategies for addressing this issue might be produced by understanding how HIF-1 α functions in cartilage and its possible biological processes. HIF-1 α can induce cartilage regeneration by controlling how genes are expressed during transcription, controlling the BMSCs in vitro cell adhesion route, activating the protein-serine-threonine kinase (AKT) pathway and the HIF1 α /FAK signaling pathway, etc. There is increasing evidence that HIF-1 α is closely associated with cartilage regeneration, such as maintaining cartilage proliferative activity, regulating chondrocyte apoptosis, promoting extracellular matrix synthesis and stimulating angiogenesis and remodelling. To more convincingly demonstrate how HIF-1 functions in cartilage regeneration, future research should aim at model experiments at the cellular-molecular level, animal model experiments and the factors, which is charge of controlling HIF-1 α , which will facilitate the translation of these experimental data into clinical applications. However, because HIF-1 α can induce angiogenesis, while providing blood supply to cartilage tissue, excessive angiogenesis may also provide blood supply for tumour production. When performing HIF-1 α -induced cartilage regeneration, care should be taken to specify the target cartilage and avoid overexpression of HIF-1 α .

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