

## Research Status of HIF-1 $\alpha$ at Cartilage Level

Yuanyuan Chu <sup>1, †</sup>, Yaobing Lu <sup>2, †</sup>, Chuning Shi <sup>3, †</sup>, Ge Song <sup>4, \*, †</sup>

<sup>1</sup> Department of Sports and Health, Bangor University, Bangor, UK

<sup>2</sup> School of Rehabilitation Medicine, Nanjing Medical University, Nanjing, China

<sup>3</sup> School of the Second Clinical, China Medical University, Shenyang, China

<sup>4</sup> School of Acupuncture and Trauma, Hubei University of Chinese Medicine, Wuhan, China

\* Corresponding author: zj071521@scccncce.cn

<sup>†</sup>These authors contributed equally to this work.

**Abstract.** Regulation of hypoxic cell survival and function requires adaptation to hypoxia, which is primarily mediated by hypoxia-inducible factor-1 $\alpha$ (HIF-1 $\alpha$ ). Cartilage and chondrocytes are exposed to a physiological hypoxic environment and studies have revealed that HIF-1 $\alpha$  primarily involves in chondrocyte survival, cartilage metabolism, cartilage osteogenesis and maintenance of chondrocyte homeostasis as well as cartilage disease. HIF-1 $\alpha$  is a cellular oxygen receptor that detects changes in cellular oxygen levels in an acute manner. HIF-1 $\alpha$  can regenerate chondrocytes among patients with osteoarthritis. Moreover, the fibrous ring and nucleus pulposus in the intervertebral disc can be renovated by HIF-1 $\alpha$ . In this paper, activation of HIF-1 $\alpha$  through mechanical factors and metal ions is discussed. Its ability of boosting oxygen levels in hypoxic surroundings and decreasing them in enriched conditions is reviewed, which is carried out via some downstream genes. In addition, participation of HIF-1 $\alpha$  in chondrocyte survival, cartilage metabolism, cartilage osteogenesis and maintenance of chondrocyte homeostasis as well as cartilage disease is introduced.

**Keywords:** HIF-1 $\alpha$ , Cartilage Tissue, Skeletal Diseases, Osteoarthritis.

### 1. Introduction

HIF-1 $\alpha$  is an important transcription factor regulating oxygen homeostasis. Identifying more than 130 HIF target genes, including cellular energy metabolism, ion metabolism, angiogenesis, erythropoiesis, apoptosis and proliferation, cell adhesion and inflammation [1]. The HIF-1 $\alpha$  mechanism betides not just during normal embryonic development and under pathologic conditions, by way of example, cancer and ischemia, and plays an important role in the development of ischemic and hypoxic diseases and tumours. HIF-1 $\alpha$  is a heterodimer comprising of subunits an alpha and a beta, HIF-1 $\alpha$  and HIF-1 $\beta$  are both bHLH/PAS family proteins, both containing the bHLH structural domain, the PAS structural domain, and the C-terminal trans-activated structural domain. HIF-1 $\beta$ , also known as the aromatic hydrocarbon receptor nuclear transporter protein, is a common subunit of many bHLH protein transcription factors and is associated with stabilization of HIF-1 and its dimer formation. HIF-1 $\alpha$  is the functional subunit of HIF and the function and activity of HIF-1 is regulated through HIF-1 $\alpha$ . The protein expression, degradation and activity of HIF-1 $\alpha$  are regulated by a variety of factors and mechanisms including hypoxia regulation, mechanical regulation, metal ion regulation and cytokine regulation, with oxygen being the most critical factor. Cartilage and chondrocytes are in a physiological hypoxic environment and HIF-1 $\alpha$  take part in normal cartilage growth and development, cellular energy metabolism, and cartilage disease, but the exact mechanisms are not yet fully understood. In this review, the contribution of HIF-1 $\alpha$  in chondrocyte survival, cartilage metabolism, cartilage osteogenesis and maintenance of chondrocyte homeostasis is summarized, as well as the function of HIF-1 $\alpha$  in cartilage diseases, including osteoarthritis, osteoporosis and intervertebral disc degeneration. The mechanism of HIF-1 $\alpha$ , the development of HIF-1 $\alpha$  in cartilage and cartilage diseases and the clinical therapeutic agents are reviewed.

## 2. Regulation of HIF-1 $\alpha$

### 2.1. Oxygen regulation of HIF-1 $\alpha$

Hypoxia-inducible factor-1 $\alpha$ (HIF-1 $\alpha$ ) expression and activity are controlled by a number of variables and mechanisms, with oxygen playing a key role. Under normoxia the half-life of HIF-1 $\alpha$  is extremely short and its stability and activity under normoxia. But under hypoxia, the stability and transcriptional activity of HIF-1 $\alpha$  transcriptional activity increased. Under normoxia, there are two main oxygen dependent pathways that inhibit HIF-1 $\alpha$  activity: the first pathway is the HIF-1 pathway, which is a JmjC structural domain 2-oxo substituted glutamate and Fe(II)-dependent oxygenase [2]. The transcribing processes of HIF-1 $\alpha$  is restrained by HIF. HIF-1 hydroxylates the aspartate residue at position 803 in the C-terminal HIF-1 $\alpha$  structural domain transactor, thereby reducing the interaction between HIF-1 $\alpha$  and the transcriptional co-activator p300/CBP to exert its inhibitory effect. The second pathway is the prolyl hydroxylase(PHD) pathway. PHD is a proline hydroxylase that stimulates the deterioration of HIF-1 $\alpha$ . PHD hydroxylates the proline residues at positions 564 and 402 within the oxygen-dependent structural domain of HIF-1 $\alpha$ . Afterwards in turn facilitates the interaction between HIF-1 $\alpha$  and pVHL for rapid degradation, then via the pVHL-regulated ubiquitin-linked protease complex pathway. HIF-1 $\alpha$  is protected from degradation under hypoxia condition and activates the expression of downstream genes by binding to HIF- $\beta$  and specific DNA sequences (HRE) in hypoxia-regulated genes. Hypoxia activates HIF-1 $\alpha$ , but recent studies suggested that prolonged hypoxia can decrease HIF-1 $\alpha$  [3]. Prolonged hypoxia increases reactive oxygen species (ROS), which induces PHD2 and HIF-1 expression through Ref-1, thereby inhibiting HIF-1 $\alpha$  expression.

### 2.2. Non-oxic regulation of HIF-1 $\alpha$

#### 2.2.1. Mechanical regulation of HIF-1 $\alpha$

In addition to hypoxia, mechanical factors can also activate HIF-1 $\alpha$ . In arterial atherosclerosis, the high distribution of low aortic arch inward curvature shear stress can enhance HIF-1 $\alpha$  through a dual process: the nuclear factor NF- $\kappa$ B-dependent induction of HIF-1 $\alpha$  mRNA and deubiquitiny lase Cezanne-dependent HIF-1 $\alpha$  protein stabilization by ubiquitin editing [4]. Although HIF-1 $\alpha$  is normatively activated in response to hypoxia, exposed to normoxic conditions, HIF-1 $\alpha$  can also be activated by mechanical shear stress, and there is a functional interaction between the mechanical response and hypoxia. The activation of hypoxic signaling by HIF-1 $\alpha$  is strengthened when cells are exposed to low shear stress.

#### 2.2.2. Metal ion regulation

Under normal oxygen conditions, metal ions Co<sup>2+</sup> and Ni<sup>2+</sup> can stabilize HIF-1 $\alpha$  to activate and induce the expression of its downstream genes, the possible mechanism is that Co<sup>2+</sup> and Ni<sup>2+</sup> replace Fe<sup>2+</sup> to combine with heme, so that hemoglobin is in a hypoxic state, and the simulated hypoxic environment stabilizes and activates HIF-1 $\alpha$ . On the other hand, as Fe<sup>2+</sup> is a cofactor of PHD, it cooperatively participates in HIF-1 $\alpha$ . However, experimental studies show that Co<sup>2+</sup> can inhibit the activity of PHD, leading to HIF-1 $\alpha$  degradation decreases and aggregates [5].

### 2.3. Regulation of cytokines

When chondrocytes are stimulated by external factors, their growth factors combine with intracellular tyrosine kinase receptors to trigger a series of intracellular signal pathways, including applying vascular endothelial growth factor (VEGF) to phosphatidylinositol 3-kinases (PI3K) and MAPK (ERK1/ERK2) signal pathways [6]. In Hep3B cells, nuclear factor  $\kappa$  B (NF- $\kappa$  B) participates in HIF-1 regulated erythropoietin gene expression through PI3K/Akt/NF- $\kappa$  B-mediated HIF-1 $\alpha$  to improve HIF-1 $\alpha$  mRNA expression level [7]. Overexpression of pVHL in cardiomyocytes causes HIF-1 $\alpha$  reduced expression. After pVHL gene knockout, HIF-1 $\alpha$  protein expression level increase, and the p38/MAPK inhibitor SB203580 can reduce the expression of pVHL, suggesting that

p38/MAPK participates in HIF-1 $\alpha$  by regulating the expression of pVHL regulation of expression. In addition, HIF-1 $\alpha$  is also regulated by physical factors, NO, CO and other factors [8].

### **3. HIF-1 $\alpha$ and cartilage cells**

#### **3.1. HIF-1 $\alpha$ regulation on chondrocyte survival and differentiation**

HIF-1 $\alpha$  is involved in inducing chondrogenic differentiation of human bone marrow cells. Zhang et al. suggested that HIF-1 $\alpha$  was a positive regulator of SOX9, and this regulatory mechanism was essential for chondrocyte differentiation and chondrogenesis [9]. In order to explore the effect of HIF-1 $\alpha$  on osteogenic and chondrogenic differentiation of bone morphogenetic protein 2-induced stem cells, Zhou et al. found that HIF-1 $\alpha$  significantly enhanced the chondrogenic differentiation of bone morphogenetic protein 2-induced stem cells, while inhibiting osteogenic differentiation and endochondral ossification, which could maintain the chondrogenic differentiation phenotype [10]. HIF-1 $\alpha$  can direct the differentiation of chondrogenic potential cells into chondrocytes. Adipose-derived adult stromal cells (ADAS) are stem cells with chondrogenic potential [11]. ADAS lacking HIF-1 $\alpha$  cannot differentiate into chondrocytes. However, other factors besides HIF-1 $\alpha$  can also affect the differentiation of ADAS cells into chondrocytes, such as the role of primitive grandmother cells, monolayer culture or aggregate culture, and the presence or absence of transforming growth factor and dexamethasone, sometimes with opposite results. For instance, hypoxia can inhibit the differentiation of ADAS into chondrocytes, but promote the differentiation of bone marrow stem cells into chondrocytes. Therefore, the effect of HIF-1 $\alpha$  on stem cell chondrogenesis is also affected by the source of stem cells and external factors, and more studies are needed to reveal its regulatory mechanism. Amir M S et al. found that in bone homeostasis regulated by bone morphogenetic protein (BMP), BMP9 inhibited PHD-mediated protein degradation under normal conditions and increased osteoblasts protein expression of HIF-1 $\alpha$ . This finding means that BMP9 can effectively increase the expression of the HIF-1 $\alpha$  protein in combination with hypoxic conditions [12].

From embryonic cartilage matrix to mature cartilage growth plate and articular cartilage, chondrocytes are always in a hypoxic environment, HIF-1 $\alpha$  is necessary for the survival and growth of chondrocytes under hypoxia. HIF-1 $\alpha$  maintain chondrocyte phenotype and endochondral homeostasis by regulating type II collagen, glucose transport matrix, VEGF, SOX9, erythropoietin and other genes. HIF-1 $\alpha$  gene can protect endplate chondrocytes in hypoxia environment and inhibit the apoptosis of endplate cartilage. When HIF-1 $\alpha$  is inhibited the expression of cartilage specific gene type II collagen, proteoglycan and SOX9 decreased during gene expression.

#### **3.2. HIF-1 $\alpha$ regulation on chondrocyte metabolism**

Generally, synthesis and decomposition of matrix in chondrocytes occur simultaneously, and mitochondria in chondrocytes are rare. Anaerobic glycolysis is the main way for endplate chondrocytes to maintain cell energy metabolism under hypoxia. Further research confirms that HIF-1 $\alpha$  regulates the expression of type II collagen and proteoglycan in chondrocytes through downstream factor SOX9, and then regulates the anabolism of chondrocytes.

### **4. HIF-1 $\alpha$ and cartilage repairment**

In tissue engineering, copper ion implantation, gene knockdown or overexpression, tissue engineering bone processing and drug addiction can all increase HIF-1 $\alpha$  expression to a certain extent. The most common way to activate HIF-1 $\alpha$  is to use PHD inhibitors, including CoCl<sub>2</sub>, DFO, DMOG, L-mim, etc. In addition to protease inhibitors, there is histone methyltransferase activation.

## 5. HIF-1 $\alpha$ and bones

### 5.1. HIF-1 $\alpha$ and fracture healing

HIF-1 $\alpha$  affects the role of osteoblasts during fracture healing. Yuanye T et al. observed HIF-1 $\alpha$  and expression of myotrophin-1 (CT-1) during fracture healing in mice undergoing mandibular osteotomy Role in osteoblasts and osteoclasts. HIF-1 $\alpha$  was found to act on osteoblastic formation and enhancement of osteoclast-mediated bone marrow mesenchymal stem cells (BMSCs) during fracture healing through CT-1 [13]. During fracture healing, not only HIF-1 $\alpha$  promote osteoclasts and osteoblasts, but also osteoclast-specific HIF-1 $\alpha$  will delay bone healing in conditioned knockout mice, reducing the chance of delayed bone healing.

### 5.2. HIF-1 $\alpha$ and bone growth

NSD1 can increase the potential of HIF-1 $\alpha$  to treat bone growth defects and fracture healing disorders caused by cartilage-derived differentiation injury. In the study of fracture repair and bone growth surface, nuclear receptor with SET domain protein 1(NSD1) can directly activate HIF-1 $\alpha$  expression, and HIF-1 $\alpha$  can regulate Sox9 expression in provocative differentiation, while Sox9 is a functional target gene for NSD1. NSD1 is regulated by Histone H3 lysine 27 trimethylation modifier (H3K36me1) in the Sox9 promoter region and H3K36me2 levels regulate its expression [14]. H3K36me1 regulates bone marrow mesenchymal stem cells (BMSCs) in addition to regulating NSD1. The study suggested that H3K27me3 collaborates with Long non-coding RNA Tmem235 (LncRNA Tmem235) promoter DNA methylation to stimulate apoptosis of BMSCs. Zhang F et al. found that in the absence of oxygen, not only HIF-1 $\alpha$  regulates Sox9 affecting H3K27me3, but also Zeste homologue 2 (EXH2) promotes modification of H3K27me3. Under hypoxia environment, LncRNA facilitated by alteration of the HIF-1 $\alpha$  and EZH2 signaling axis modification of the Tmem235 promoter H3K27me3, leading to BMSC apoptosis [15]. Therefore, under hypoxic environment of BMSC transplantation, the apoptosis rate of BMSC can be reduced by controlling H3K27me3 and LncRNA Tmem235 promoter DNA methylation.

### 5.3. HIF-1 $\alpha$ and skeletal diseases

HIF-1 $\alpha$  is essential for the maintenance of cartilage homeostasis. In recent years, the role of HIF-1 $\alpha$  in cartilage diseases has become a research hotspot. The balance between extracellular matrix synthesis and metabolism is broken in patients with osteoarthritis. The pathological change of osteoarthritis is a process of decreased resistance of articular chondrocytes to external pressure, accompanied by decreased proliferation and differentiation ability of chondrocytes, energy metabolism and responsiveness to growth factors. Cartilage necrosis in the pathological mechanism of osteoarthritis is not only related to the loss of extracellular matrix, but also closely related to chondrocyte death. Apoptosis and necrosis of chondrocytes are involved in the pathological process of osteoarthritis. Cartilage injury in osteoarthrosis is mainly caused by the death of chondrocytes mediated by catabolic enzymes (apoptosis and autophagy). For example, inflammatory mediators are involved in the pathological process of osteoarthritis, including IL-1, IL-6, TNF- $\alpha$ , proteolytic enzymes, and MMP aggregation to cause cartilage injury. Tan et al. studied the regulation of HIF-1 $\alpha$  by IL-1 $\beta$  and found that IL-1 $\beta$  upregulated the post-transcriptional level of HIF-1 $\alpha$  protein in human osteoarthritis chondrocytes. In comparison with the joint cartilage of healthy people, the articulation of HIF-1 $\alpha$  and VEGF in joint cartilage of patients with osteoarthritis was significantly increased, indicating that HIF, VEGF and myocyte enhancing factor-2C all take part in the pathogenesis of osteoarthritis, and the increase of HIF-1 $\alpha$  was positively correlated with the expression of VEGF. Collectively, it is suggested that HIF-1 $\alpha$  and VEGF play a joint role in the pathogenesis of osteoarthritis.

Besides osteoarthritis, HIF-1 $\alpha$  and VEGF also participate in rheumatoid arthritis(RA). In the experiments of Fattah S, A et al., divided into 100 healthy people and 100 RA patients, found that in RA the YWHAH gene (gene mutations occurring in RA) and its expressed protein 14-3-3 $\eta$  increased

by HIF-1 $\alpha$  and VEGF. Then expressed protein 14-3-3 $\eta$  promote the development of RA [16]. By impacting skeletal diseases, HIF-1 $\alpha$  can activate VEGF, protein 14-3-3 $\eta$  and other factors and controlled by antioxidant. Resveratrol (RES) as an antioxidant can promote the production of osteoblasts and inhibit the formation of osteoclasts, which can prevent osteoporosis caused by altitude hypoxia. In the experiments of Yan C et al., male rats received RES (400 mg/kg) once daily for nine weeks in hypoxic surroundings to observe in vivo and in vitro changes. The study found that RES promotes osteoblast production and improves alkaline by inhibiting Reactive oxygen species and HIF-1 $\alpha$  in vitro. And improve hosphatase, osteocalcin and osteopontin levels, prevent osteoporosis [17]. Most studies are all about the influence of HIF1 $\alpha$  on cartilage diseases in bone. In addition to, Zhenyu Jin et al. showed the influence of HIF-1 $\alpha$  on intervertebral disc. It is found that HIF-1 $\alpha$  taken effect in degenerative disc regeneration [18]. Specifically in the development and homeostasis of the nucleus pulposus, HIF-1 $\alpha$  participates in various processes of disc degeneration, promotes the disc extracellular matrix in the nucleus pulposus, maintains the metabolic activity of the nucleus pulposus, and regulates the dystrophy mineralization of the nucleus pulposus.

## 6. Clinical drugs

Nifedipine is a well-known antihypertensive drug and L-type calcium channel inhibitor, while studies have shown that HIF-1 $\alpha$  as well as genes related to cartilage growth can be strongly upregulated when a certain amount of nifedipine is taken under hypoxia [19]. Nifedipine can inhibit the proliferation of chondrocytes of cartilage origin alone under hypoxic conditions and reduce intracellular calcium in the medium of cartilage differentiation, but has no significant effect on chondrocytes of multiple cartilage sources. In hypoxic conditions, nifedipine inhibits chondrocyte proliferation and differentiation by upregulating the cartilage gene collagen I (COL2A1) and aggregated glycans (ACAV). At the same time, HIF-1 $\alpha$  upregulated by nifedipine can also affect COL2A1 and ACAN, which in turn affects the proliferation of chondrocytes.

## 7. Discussion

With more and more scholars studying cartilage and HIF-1 $\alpha$  in depth, it is becoming clearer and clearer that HIF-1 $\alpha$  is involved in the physiological and pathological regulation of cartilage. More signaling pathways have been found to be involved in regulating chondrocyte survival and metabolism, and HIF-1 $\alpha$  also has a hand in these signaling pathways, but the interaction mechanism between multiple signaling pathways is not yet clear. The treatment of osteoarthritis and the repair of cartilage damage are still clinical problems, and a large number of studies are exploring ways to overcome this problem. The application and research of cartilage damage repair and prevention in plastic repair and rehabilitation is also a novel direction of thinking.

## 8. Conclusion

With the penetrative study of cartilage and HIF-1 $\alpha$  by scholars, its role in regulating cartilage physiology and pathology has become increasingly evident. HIF-1 $\alpha$  partakes in multiple signaling pathways that have been found to be involved in the regulation of chondrocyte survival and metabolism. However, the interaction mechanism between these signaling pathways remains unclear. The treatment of osteoarthritis and repair of cartilage damage are still clinical challenges, and a large number of studies are exploring ways to overcome this problem. Current studies have suggested that HIF-1 $\alpha$  plays a part in protecting chondrocytes in osteoarthritis. At present, HIF-1 $\alpha$  is believed to participate in protecting chondrocytes in osteoarthritis. Studying the role of HIF-1 $\alpha$  in cartilage tissue regeneration will provide new ideas for the treatment of cartilage injury. In the future, researchers can focus on the mechanism of synergistic interaction between HIF-1 $\alpha$  and other pathways to play a more than double role and improve the speed of cartilage repair.

## References

- [1] Clottes E. Hypoxia-inducible factor-1: regulation, involvement in carcinogenesis and target for anticancer therapy. *Bull Cancer*, 2005, 92 (2): 119 - 127.
- [2] Yue W, Zhihong L, Michael AM, et al. Inhibition of the oxygen-sensing asparaginyl hydroxylase factor inhibiting hypoxia-inducible factor: A potential hypoxia response modulating strategy. *J. Med. Chem.* 2021, 64, 11, 7189 - 7209.
- [3] Kobayashi Y, Oguro A, Imaoka S. Feedback of hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) transcriptional activity via redox factor-1 (Ref-1) induction by reactive oxygen species (ROS) [J]. *Free radical research*, 2021, 55 (2): 154 - 164.
- [4] Feng S, Bowden N, Fragiadaki M, et al. Mechanical activation of hypoxia-inducible factor 1 $\alpha$  drives endothelial dysfunction at atheroprone sites [J]. *Arteriosclerosis, thrombosis, and vascular biology*, 2017, 37 (11): 2087 - 2101.
- [5] Epstein AC, Gleadle JM, Mcneill LA, et al. *elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation [J]. *Cell*, 2001, 107 (1): 43 - 54.
- [6] Fukuda R, Hirota K, Fan F, et al. . Insulin-like growth factor 1 induces hypoxia-inducible factor 1-mediated vascular endothelial growth factor expression, which is dependent on MAP kinase and phosphatidylinositol 3-kinase signaling in colon cancer cells [J]. *J Biol Chem*, 2002, 277 (41): 38205 - 38211.
- [7] Figueroa YG, Chan AK, Ibrahim R, et al. NF-kappaB plays a key role in hypoxia-inducible factor-1-regulated erythropoietin gene expression [J]. *Exp Hematol*, 2002, 30 (12): 1419 - 1427.
- [8] Teng M, Jiang X, Zhang Q, et al. Microtubular stability affects pVHL-mediated regulation of HIF-1 $\alpha$  via the p38 /MAPK pathway in hypoxic cardiomyocytes [J]. *PLoS One*, 2012, 7 (4): e35017.
- [9] Zhang C, Yang F, Comelia R, et al. Hypoxia-inducible factor 1 a positive regulator of Sox9 activity in femoral head osteonecrosis [J]. *Bone*, 2011, 48 (3): 507.
- [10] Zhou N, Huang W, Liao J, et al. Effect of hypoxia inducible factor small a on chondrogenic and osteogenic differentiation of stem cells induced by bone morphogenetic protein. *Journal of third military medical university*, 2014, 30 (12): 1243 - 248.
- [11] Malladi P, Xu Y, Chiou M, et al. Hypoxia inducible factor-1 alpha deficiency affects chondrogenesis of adipose-derived adult stromal cells[J]. *Tissue Eng*, 2007, 13 (6): 1159 - 1171.
- [12] Amir M S, Chiba N, Seong C H, et al. HIF-1 $\alpha$  plays an essential role in BMP9-mediated osteoblast differentiation through the induction of a glycolytic enzyme, PDK1 [J]. *Journal of Cellular Physiology*, 2022, 237 (4): 2183 - 2197.
- [13] Yuan T, Qi S, Yi T, et al. HIF-1 $\alpha$  regulates osteoclast activation and mediates osteogenesis during mandibular bone repair via CT-1. *Oral Diseases*, 2022, 28 (2): 428 - 441.
- [14] Shao, R., Zhang, Z., Xu, Z. et al. H3K36 methyltransferase NSD1 regulates chondrocyte differentiation for skeletal development and fracture repair. *Bone Res* 9, 30 (2021).
- [15] Zhang F, Luo H, Peng W, et al. Hypoxic condition induced H3K27me3 modification of the LncRNA Tmem235 promoter thus supporting apoptosis of BMSCs[J]. *Apoptosis*, 2022, 27 (9): 762 - 777.
- [16] Fattah S A, Fattah M A A, Mesbah N M, et al. YWHAH Genetic Variants are Associated with Increased Hypoxia Inducible Factor-1 $\alpha$ /Vascular Endothelial Growth Factor in Egyptian Rheumatoid Arthritis Patients [J]. *Biochemical Genetics*, 2022: 1 - 14.
- [17] Yan C, Wang Z, Liu W, et al. Resveratrol Ameliorates High Altitude Hypoxia-Induced Osteoporosis by Suppressing the ROS/HIF Signaling Pathway [J]. *Molecules*, 2022, 27 (17): 5538.
- [18] Kim J W, Jeon N, Shin D E, et al. Regeneration in Spinal Disease: Therapeutic Role of Hypoxia-Inducible Factor-1 Alpha in Regeneration of Degenerative Intervertebral Disc[J]. *International Journal of Molecular Sciences*, 2021, 22 (10): 5281.
- [19] Bironaite D, Miksiunas R, Bernotiene E, et al. The effect of nifedipine on human chondrocytes from osteoarthritic cartilage cultivated under normoxic and hypoxic conditions. *Osteoarthritis and Cartilage*, 2021, 29: S134 - S136.