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The Role and Regulation Factors of HIF-1A in Bone Regeneration

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Abstract:

As one of the most common diseases, bone injury has shown an upward trend during decades. Recent research have shown much more focus on regulating bone homeostasis and it details homeostasis and other pathways to accelerate initial recovery, which has a positive effect on both injuries and further treatment. This review discussed how HIF-1A dual-directionally regulates two important bone-related cells-osteoclasts and osteoblasts. and osteoblasts. It was found that HIF-1A could regulate cells through various pathways, including affecting their progenitor cells, metabolic pattern and HIF-1A downstream genes. This paper also discussed the regulation pathway of HIF-1A on both chemical and physical leading signals. The mechanical factors like substrate stiffness or shear stress have been studied that could regulate HIF-1A activity. Research has also found the effect of extracellular pH on HIF-1A activity through its two degradations PHD and VHL. This review summarized the HIF-1A effect on bone homeostasis with the regulatory pathways that could regulate its activity. This review also discusses the possible future research direction of how HIF-1A regulates its downstream genes and further influences hypoxia condition. It provides some valuable ideas for the development of this rapidly developing field.

Keywords: Hypoxia inducible factor-1A; bone regeneration; bone homeostasis; hypoxia

1. Introduction

The rise in population and increased amounts of sports injuries, natural disasters and traffic accidents have led to a higher risk of injury [1]. Particularly in sports fields like snowboarding, skateboarding, and hockey. Both osteoblasts and osteoclasts are essential for bone homeostasis and bone repair in normal physiological settings. [2]. The function of osteoclasts is the mechanism that induces bone resorption and remove dead osteocytes and regulate blood calcium levels [2]. Conversely, osteoblasts functioning as a key bone formation factors. Bone marrow mesenchymal stem cells (BMSCs) and bone marrow derived macrophages (BMMs) are osteoblasts‘ and osteoclasts‘ progenitor cells [3]. However, both BMSCs and BMMs can be affected by extracellular conditions, such as low oxygen concentration or upstream gene expression, which can lead to infection at the injury site, slow recovery, or even a direct risk of deterioration into osteoporosis [3, 4]. Many bone injury sites are often in a state of hypoxia. Therefore, it is important to understand the key points of bone defect recovery to promote bone formation [2].

Hypoxia inducible factor 1A (HIF-1A) is a significant transcription factor that reacts to low oxygen levels, its expression correlates with oxygen degree [5]. Under normoxia, HIF-1A degradation are correlated with proline hydroxylase (PHD) and von Hippel-Lindau protein (VHL) [6, 7]. Yet, the breakdown process is inhibit under hypoxic environments, HIF-1A would then activates it hypoxia response related downstream genes [8, 9]. The efficiency of HIF-1A to change the hypoxia condition and increase nutrients is considerable due to its ability to activate a series of angiogenesis-related factors such as VEGF in the upstream and it can regulate more than 100 downstream genes, which has more pathways compared to only activating VEGF, and it is positive for bone regeneration [10]. HIF-1A can also affect bone metabolism by regulating cellular metabolism. Under hypoxic conditions, HIF-1A can promote glucose catabolism and lactate production to satisfy energy needs by regulating multiple metabolic pathways, such as the respiratory chain of the mitochondria, the TCA cycle, and glycolysis. Additionally, it promotes apoptosis and protects against oxidative stress. The biological role of HIF-1A in orthopedics fields has discovered in detail over the past few decades. This review summarizes the role of HIF-1A in bone defect, looks to the reasons why the regeneration pathway is highly related to HIF-1A, and discusses existing comment pathways for regulating HIF-1A.

2. The Role of HIF-1A in Bone Defect Recovery

2.1 The Role of Osteoclasts and Osteoblasts in Bone Homeostasis

Osteoclast, a bone-resorbing cell, is responsible for bone

resorption to degrade dead or dysfunctional cells in bone tissue [11]. When osteoclasts differentiate from their precursor cells BMMs, by secreting acids (H+), proteases (like CTSK), and matrix metalloproteinases (MMPs), they will resorb bone matrix. Additionally, they will construct a tight connection between the basement membrane of osteoclasts and the bone surface, forming a sealed compartment to resorted bone matrix efficiently [3, 11, 12]. Osteoclast has developed a specialized cytoskeleton and cytoplasmic lysosomes that allow it does this by creating a separate microenvironment between itself and the bone, where a wide range of hydrolytic and proteolytic enzymes released by the lysosome and acid cause matrix destruction [2, 11–13]. However, the function of how osteoclasts engulf extracellular particulars and get them inside their cytoplasmic lysosomes is still unexplored, it needs further exploration to fully understand the full resorption process and special uptake function of it.

Osteoblast is a bone formation-related bone cell, and the precursor cells of it are BMSCs [3]. Osteoblast highly correlates with osteoclast, and it will terminally differentiate into osteocytes which represent more than 90% of adult bone cells, showing its crucial role in bone regeneration [14]. In the process of mature osteoblast differentiation into osteocyte, the osteoblast first contacts with the osteocyte layer and mineralizes part of itself to turn into a progenitor osteocyte. After it mineralizes and keeps contact with the osteoblasts layer, it keeps migrating to the osteocyte layer and terminally mineralizes into osteocytes. Remarkably, this precursor osteocyte-osteoblast keeps contact with neighboring cells, the osteoblast layer, and the blood vasculature system under the differentiation process [2, 11]. Palumbo et al. [2] analyze the process of bone homeostasis and claim that osteocytes play an “operation center” in the whole process, it brings the research into a novel aspect that researchers have hardly ever explored [2]. The relationship between osteocyte and osteoblast communication still needs further exploration, to have further understanding and utilization of this novel idea and help the bone defect recovery with the applications.

2.2 The Function of HIF-1A in Dual-directionally Regulates Osteoclast and Affect Osteoblasts in Hypoxia Condition

The bone injury environment requires a lot of energy to produce new bone cells and accelerate bone homeostasis, resulting in a repair environment generally affected by hypoxic and nutrient deficiency, it is a primary factor that decreases the rate of bones heal [4]. HIF-1A is a important regulator of hypoxic condition, with more than 100 downstream hypoxia-regulated genes, and is associated with osteoblasts and osteoclasts activity regulation [10, 15].

Previous research has revealed that under different extracellular condition, HIF-1A would have different effects on the activity of osteoclasts and osteoblasts [16]. It will activate or inhibit bone homeostasis, and finally fasten bone regeneration or cause serious infection or osteoporosis. Research shown that HIF-1A could activates osteoclasts‘ resorption process by promoting c-Jun N-terminal kinase (JNK) route to stimulate osteocyte-producing RANKL and VEGF to trigger BMMs differentiation pathway, thus creating osteoclasts starting the resorption and initiate bone regeneration process. In fact, almost every bone regeneration is observed at the site where bone resorption has occurred [17]. It shows researchers that osteoclasts are essential for initiating the process of bone regeneration, instead of stopping or inhibiting bone regeneration. Studies show that HIF-1A could activate osteoclasts to secrete cardiotrophin-1 (CT-1), an IL-6 superfamily gp130 signaling cytokine, it would induce BMSCs to differentiate and terminally increase the number of osteoblasts [18]. Contrary, HIF-1A could inhibit osteoclast activity by inducing osteoblasts to secrete osteoprotegerin (OPG), which is a receptor that can inhibit osteoclast resorption [16]. Results have showed HIF-A regulates osteoclast and osteoblasts activity in two different ways through serval pathways. However, Chen et al [19] have found that HIF-1A can promote bone regeneration at the macro level by activating BMSCs osteogenesis differentiation and breaking BMMs differentiation by knocking out the VHL gene, which is a crucial HIF-1A degradation gene [7]. This suggests that the detailed mechanisms of the interactions and associations between HIF-1A and osteoblasts osteoclasts still need to be further investigated and quantitatively analyzed experimentally are needed, to explore the link between macro-performance and micro-pathways, and the overall effect of the superimposition of different response pathways.

2.3 The Degree of Hypoxia Influences HIF-1A Expression and Function as well as BMSC Osteogenic Activity.

Related studies have discussed the activity and induction aspect of HIF-1A under different oxygen condition. Hypoxia is classified into three degrees according to the oxygen level: severe hypoxia, moderate hypoxia, and mild hypoxia (1% O2, 2% O2, and 5% O2, respectively).

Under 5% oxygen level, HIF-1A usually has a very short half-life. However, according to current research, HIF-1A can boost BMSC differentiation and proliferation in the same hypoxic environment. [20]. It also shows the potential to differentiate into osteoblast, chondrocyte or adipocyte, which can enhance the effectiveness and process of bone regeneration. In contrast, hypoxia will inhibit HIF-1A‘s action in BMSCs under 1-2% oxygen degree by activating the Notch1 signal pathway to inhibit PBMSCs osteogenesis differentiation, it shows a dual effect of hypoxia condition on the activity of HIF-1A [21]. Research

has also shown when the duration of the hypoxia condition keeps extending, HIF-1A expression would drop while CbfA1 expression would rise, turn out to increase the process by which BMSCs develop into osteoclasts and chondrocytes. This would eventually have negative effects on bone regeneration [22]. Notably, studies show that under 0.2% oxygen degree, the environment will upregulate C/EBPs and HIF-1A, but it would activate BMSCs to differentiate into adipocytes instead of osteoblasts.

Those studies have shown that different oxygen levels have a bidirectional effect on HIF-1A, which in turn impacts BMSCs‘ osteogenic differentiation. Meanwhile, prolonged hypoxia reduces the expression of HIF-1A in BMSCs have been shown in recent studies, suggesting that the use of oxygen concentration to regulate HIF-1A and thus osteogenic activity is subject to a number of limitations, and that these mechanisms need to be further investigated to stabilize the control and finally could be applied to clinical.

2.4 HIF-1A Regulated Metabolic Pattern Changes

The downstream gene of HIF-1A can alter bone metabolism patterns by regulating cellular metabolism. HIF-1A controls the transcription of numerous genes, including lactate dehydrogenase (LDHA), glycolytic enzymes (such as 1,2-hexose kinase, phosphoglycerate kinase 1, and pyruvate kinase M2), and glucose transporters (GLUT1 and GLUT3), are involved in the metabolism of glucose. HIF-1A mediates the transcription of multiple genes involved in glycolysis under hypoxic conditions. As a result, HIF-1A promotes glucose catabolism and accelerates energy production to varying degrees compared to hypoxic environments that lack HIF-1A regulation [6].

Specifically, HIF-1A changes the metabolic pattern to create ATP and utilize energy much more efficiently through its metabolism-related downstream genes in hypoxic conditions. Under normoxia, aerobic glycolysis is typically preferred in cellular metabolism, which is carried out in mitochondria. However, when the expression of HIF-1A stabilized through inhibit degradation process, the cellular metabolism would change to anaerobic glycolysis, which could save oxygen for other bone regeneration and recovery processes. Additionally, HIF-1A would halt the TCA cycle and OxPho to lower mitochondrial oxygen consumption. Although glucose produced to lactate instead of pyruvate, which will reduce the ATP produced per glucose molecule. But according to previous studies, HIF-1A could promote glucose catabolism and lactate production, and lactate can promote the activity of HIF-1A through positive feedback [7]. Ultimately, the amount of ATP generated could be equal to aerobic phosphorylation [15]. Notably, HIF-1A could upregulate a key enzyme PDK1 to promote phosphorylation in the hypoxia bone defect area and induce BMSCs osteogenesis differentiation and osteoblast differentiation into osteocytes, thus promoting bone regeneration [16, 23]. It has been proven that HIF-1A has a considerable effect on increasing bone mass [24].

In addition, HIF-1A can promote vascularization to change the environment’s hypoxic and nutrient-deficient condition, which could change the hypoxia condition in a long term. HIF-1A also promotes the growth of healing tissue, blood vessels and bone tissue, which are all important factors in bone regeneration and further recovery of patients [25]. In conclusion, HIF-1A has various effects on the metabolism of the bone environment, which can generally change the metabolism efficiency and positively promote osteogenesis, but the fact that this key factor is affected by many factors cannot be ignored.

3 Pathways Regulating HIF-1A

3.1 HIF-1A Regulated by Inhibit Its Two Degradation Factors

Under normoxia, HIF-1A is degraded by PHD and VHL with a half-life of only 5 minutes to prevent potential damage. Studies have shown the high possibility of using these two degradation factors PHD and VHL. Studies have discovered that controlling HIF-1A activity by inhibiting PHD or VHL could be very efficient and profitable [19, 25].

Under normoxia, after binding to and breaking down the hydroxylated HIF-1A protein, VHL thereby altering HIF-1A expression [16]. However, studies have discovered that in an acidic environment, an increase in hydrogen ions causes nucleolar sequestration of VHL, resulting in a temporary and reversible loss of function. This will affect HIF-1A activity and terminally increase its expression [13]. Research has found that under a low pH condition such as pH 6.0, the protein levels of HIF-1A have shown a statistically significant increase. Also, previous research has found that because the increase in HIF-1A protein level is due to VHL sequestration, the low pH condition would have no significant effect on HIF-1A mRNA level [13].

PHD contributes similarly to HIF-1A degradation, some PHD inhibitors can be utilized to prevent HIF-1A degradation have been confirm in previous studies, which may thereby activate HIF-1A activity. This pathway has shown a high possibility to increase fracture vascularity, and bone volume and could increase bone mass stiffness [25]. However, Komatsu et al. [26] discovered that in a mouse femur fracture model comparable to this one, partial deactivation of HIF caused an increase in the size of the fracture callus and a decrease in the rate of apoptosis, which has contrasting results compared to another research.

These contradictory show researchers that although the high profit and simpler strategies avoid the need for com-

plex reaction pathways, the detailed function and interaction between HIF-1A degrader and itself still need further experiment to truly utilize those pathways in clinical therapy and bone defect area.

3.2 HIF-1A Regulated by Physical Signaling

Apart from chemical elements, physical signaling plays a crucial role in controlling HIF-1A. Mechanical strain and substrate stiffness in the bone injury environment play a key role in regulating HIF-1A activity. Sheer stress studies have found that mechanical stretch alone, independent of hypoxic stress, can boost the amounts of HIF-1A protein by ubiquitin editing-mediated Cezanne-dependent stability of HIF-1A protein after NF-κB-dependent HIF-1A mRNA synthesis [27]. Studies of atherosclerosis have also shown that HIF-1A is elevated in response to mechanical shear stress, which is NF-κB and Cezanne-dependent in areas where atherosclerosis is most likely to occur [27]. Research on HIF-1A induction of VEGF activity also mentions the role of mechanical stress in regulating HIF-1A activity in the complex mechanical environment of the myocardium. In this myocardial regulation pathway, researchers believe that HIF-1A expression is increased via the PI3K/Akt/FRAP pathway and SACs [28].

On the other mechanical aspect, high substrate stiffness has been shown in earlier research to increase HIF-1A expression at the protein and mRNA levels [13, 29]. Studies about a drug called Tamoxifen have discovered that with the drop in substrate stiffness, the mRNA level of over 25 hypoxia and HIF-1A-related genes has been downregulated, it can further prove the regulating effect of substrate stiffness on HIF-1A activity. Moreover, the studies have also found at the mRNA level, HIF-1A could be carried out with the same protein level while showing an increase in mRNA level [29]. Some studies have also mentioned Yes-associate factors (YAP-1), One transcription factor that is crucial for interacting with HIF-1A in a complicated mechanical [29]. However, the complex matrix condition in different body regions shows researchers that there are still many side effects and the function of force-related regulation remains unclear, as the reason why there is still a big gap in the force-regulated pathway for HIF-1A.

4. Conclusion

In this review, the researcher provides the summary of HIF-1A function in bone regeneration area and the pathway to regulate it under different extracellular conditions. Studies show us that under hypoxia conditions, it is necessary to utilize HIF-1A to promote the patient‘s bone regeneration and recovery under tissue hypoxia condition. Its regulation pathway varies, due to HIF-1A control of more than 100 downstream genes, which are highly connected with angiogenesis, regulation of hypoxic environment and bone circulation. This ultimately makes it have a bi-directional regulatory effect on osteoclast and osteoblast and affect bone homeostasis. The regulation of hypoxia on HIF-1A and bone homeostasis has also been discussed. Researchers found that under different hypoxia conditions, the activity of HIF-1A would have considerable change. Remarkably, HIF-1A activity would decrease if it remained in the hypoxic state for too long. This leads the research field to focus more on short-term regulation of HIF-1A activity. It also leads to irregular changes in bone homeostasis while oxygen conditions are changing. Review has summarized serve regular way of HIF-1A with both chemical and physical factors. Research has shown the potential to regulate HIF-1A by controlling its degradation-related molecules, PHD and VHL. Using mice models, researchers have confirmed this kind of pathway ability on angiogenesis and osteogenesis-related expression. It can also increase bone mass, which is crucial for bone final recovery. Studies have shown the pH regulation ability, which is by sequestrating VHL to affect HIF-1A activity. Considering the complex mechanical stress in bone defect areas, the studies on physical signals have also been summarized. Both high substrate stiffness and high shear stress (wall stress) could positively regulate HIF-1A at both protein and mRNA levels. However, the detailed mechanism of these pathway-overlapping effects remains unclear.

Bone defect-related injuries are still highly dangerous, with the possibility of severe infection or long recovery time. With the further discovery and understanding of HIF-1A‘s role in bone homeostasis, researchers have noticed that there is still a big blind spot in the co-effect of its various downstream genes. Thus, more research is needed to clarify the long-term or short-term relationship between HIF-1A and bone homeostasis. Future research should focus on identifying the communication elements that govern HIF-1A, such as hormone and temperature, and find out the detailed reason and pathway of its bi-directional regulatory effect.

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