Mechanism of Non-Canonical Regulation Pathways of HIF-1α and Their Possible Applications in Cartilage Medical Bioengineering

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Abstract. A recent focus of research, medical bioengineering aims to advance fundamental ideas, generate knowledge from the molecular to the organ level, and create novel biological products, materials, processing techniques, implants, instruments, and informatics methods for disease prevention, diagnosis, and treatment, patient rehabilitation, and health enhancement. Cartilage damage has been a clinical troublesome for a long time. Traditional therapies have lots of limitations. Recent studies have found that under the presence of HIF-1, mesenchymal stem cells (MSCs) have the tendency to differentiate into chondrocytes. Associated with advanced bioengineering techniques, this finding provides a new aspect of cartilage repairing. This article introduces HIF-1 and HIF-1α as its regulatory subunit, the source and differentiation directions of MSC and relations between it and HIF-1, articular cartilage arthritis and normal arthritis and the mechanism of three non-canonical regulations of HIF-1α while analyze their advantages and existing issues, aiming at provides new and reliable future cartilage repairing.

Keywords: Hypoxia-Induced Factor-1 Alpha, Cartilage Repairing, Mesenchymal Stem Cell.

1. Introduction

1.1. HIF-1α

HIF1-α is a subunit of transcriptional factor HIF-1. HIF-1 acts as a central transcriptional regulator of the cellular and systemic homeostatic adaptive response to hypoxia. Under the circumstances of hypoxia, it activates the transcription of over 40 genes, which include erythropoietin, glucose transporters, glycolytic enzymes, vascular endothelial growth factor, HILPDA, and other genes whose protein products promote oxygen supply or facilitate cellular metabolic adaptation to hypoxia. HIF-1 is activated when its free α subunit binds to its DNA-binding β subunit and forms a diameter. Hypoxia significantly increases the amount of HIF-1α protein, which is necessary for HIF-1 function, although HIF-1 protein is always showing independent of oxygen tension [1].

1.2. Mesenchymal stem cell

Mesenchymal stem cells (MSCs) are stromal cells with multilineage differentiation and the capacity to self-renew. MSCs can be extracted from a wide range of tissues, including the umbilical cord, adipose tissue, menstrual blood, endometrial polyps, etc [2]. MSCs are able to differentiate to myocyte, hepatocyte, osteocyte, adipocyte, chondrocyte and etcetera other cell types. MSCs are a desirable option for the development of potential clinical applications due to their multipotent characteristics. Vitro experiments have shown that MSCs' chondrocyte direction differentiation is carried out by hypoxia and this has been proved to be activated by HIF-1 activation [3].

1.3. Articular cartilage damaged arthritis

Articular cartilage is a kind of tissue that covers on the surface of articular bone at articulatio genus, articulatio cubiti, articulatio coxae, etc. It functions as a barrier between two acral articular bones, which avoid direct contact between them and abrasion is therefore eliminated or highly reduced. However, cartilage has an enduring limitation and if it is exposed to a stress higher than the limitation or as the increasing of age, it will have a high possibility to be frayed, which will further cause

abrasion of appendicular joint and lead to arthritis. Articular cartilage can only self-renew in vivo. when its abrasion speed is faster than its regeneration in a long period, it will be worn out. Once it is completely worn out, its self-repairing becomes impossible. Therefore, arthritis and arthralgia caused by articular cartilage abrasion still remain a chronic clinical issue.

As mentioned above, if HIF-1α can be regulated by artificial conditions, MSCs will have a gigantic potential in cartilage repairing. Past in vitro experiments use hypoxia as canonical regulation of HIF-1α and induce MSCs' chondrocyte-direction differentiation. However, in real circumstances, this method has lots of limitations on applications. For example, MSCs show low mitotic activity and low survival ratio under hypoxia cultivate condition, which makes it hard to generate stable cell lines. Recently, scientists have found several non-canonical regulations of HIF-1α and its downstream genes. This article will focus on 3 typical already discovered non canonical regulations of HIF-1α, analyzing their principles and make some discussion and comments on their advantages, potentials, disadvantages and possible issues of each of mentioned regulations.

Depending on the regulatory factor's features, I will divide the following discussion into two parts. The first part is HIF-1α regulated by physical factors and the second part is HIF1-α regulated by biochemical factors.

2. HIF-1α regulated by physical factors

2.1. HIF-1α regulation by stretch-activated channels in specific tissues

A recent study by Kim et al. suggests that stretch-activated channels, the SAC/PI3K/Akt/FRAP pathway, and HIF-1 play major roles in the production of VEGF in nonischemic and mechanically stressed myocardial. Furthermore, the myocardium's ability to adapt to stressors appears to be significantly influenced by this signaling system, which activates HIF-1.

Researchers first established regional ischemic-heart rats. They then developed ex vivo stretch and in vivo hemodynamic overload models using these animals. Rats' left ventricular pressure was measured with a plastic catheter companied by a tiny balloon tip that was introduced into the left ventricle through the mitral valve in order to establish an ex vivo stretching model. Aortocaval shunt surgery (ACS) was carried out as previously reported in order to inflict constant mechanical stress on the rat myocardium [4]. On the protein level, immunoprecipitation and immunoblot analysis was adopted to exam the expression of HIF-1α protein in the ventricular myocardium, and the downstream genes VEGF and Akt were tested by immunoblot analysis. Total RNA was extracted at the RNA level using a semiquantitative reverse transcriptase-polymerase chain reaction assay. Finally, they introduced immunohistochemistry to re-target HIF-1α in myocardium tissues. Several results have been discovered through above experiments. First, the downstream gene VEGF is induced in the non-ischemic myocardium, and the situation is same in the ischemic myocardium. As one of the most important transcriptional factors of VEGF, the protein level of HIF-1α have been studied and the results showed that HIF-1α accumulates in the nuclei of myocytes in the nonischemic myocardium. Then, it is demonstrated that diastolic wall stress occurs in the ischemic heart and that mechanical stress is the factor that initiates the nuclear accumulation of HIF-1 and VEGF gene expression [5]. Finally, by silencing possible signaling pathways, this regulation is validated to be adjusted by the SAC/PI3K/Akt/FRAP pathway.

This provides a simple way of regulating HIF-1 and treating cartilage-damaged arthritis. For example, early-stage cartilage abrasion can actually be cured by a certain amount of movement. For those advanced stage cartilage abrasion, based on mechanical stress-related cartilage repairing that have been reported, a kind of micro robot which can produce constant mechanical stress can be implanted to induce cartilage self-repair [6]. However, some issues should be considered. First, the magnitude of this mechanical stress has not yet been quantified, and overloaded mechanical stress will damage cartilage on the contrary, further quantitative researches should be done. Second, further research is required to determine whether the PI3K/Akt/FRAP system, which mediates HIF-1 and

VEGF overexpression at the atmospheric oxygen tension, also acts in articular cartilage tissue as this route was previously only detected in cancer cells in the myocardium.

2.2. HIF-1α activation by mechanical low shear stress

Research done by Feng S et al. has supposed that modest shear stress induced HIF-1α in cultured endothelial cell (EC) under normoxic circumstance. The HIF-1 protein was maintained by the deubiquitinating enzyme Cezanne, which was induced by the transcription factor nuclear factor-B (NF-κB), which also induced HIF-1α transcripts.

An earlier study found that the expression of HIF-1 and some of its downstream genes incresed in the porcine aorta's atheroprone (low shear stress; inner curvature) and atheroprotected (high shear stress; outer curvature) regions [7]. Their further research demonstrates that in the presence of atmospheric oxygen, and HIF-1 is triggered by mild shear stress, which also sets up the EC for increased HIF-1 activation in response to hypoxic signaling. Modest shear atheroprone regions express HIF-1 and downstream glycolysis genes preferentially, and HIF-1 expression is sustained during early atherogenesis. The next discovery is that modest shear stress promotes HIF-1 at the mRNA level, according to qRT-PCR. RelA and p50 subunits are activated by modest shear stress in cultured EC, as demonstrated by DNA-binding enzyme-linked immunosorbent test, this is likely to be accomplished via a transcription factor of HIF-1 that responds to inflammatory signals, NF-κB [8]. This is demonstrated by overexpression of IκBα, an inhibitor of NF-κB. While the group with an expression plasmid containing IκBα showed reduced HIF-1α expression in cultured EC, the group with a blank plasmid showed no difference on HIF-1α expression level. Silencing of RelA NF-κB subunits also showed same results. And it is known that HIF1-α undergoes degradation under atmospheric oxygen tension but HIF1-α also showed elevated expression on protein level, so researchers assumed that there must be a low shear stress-induced mechanism that prevent HIF-1α from degrading. First, the effect of proteins that will destabilize HIF-1α such as PHD, von Hippel-Lindau (VHL) E3 ubiquitin ligase is tested [9]. The result showed no suppression on them. However, deubiquitinating enzyme Cezanne can stabilize HIF-1α, which is achieved by preventing its degradation [10]. Further exploration validated that for deubiquitination, Cezanne can target HIF-1 in EC under modest shear stress.

Similarly, mechanical stress is the external inducing factor of HIF-1α, the type of mechanical stress and magnitude of mechanical stress have been clarified. This provides a direction for current rehabilitation treatment. However, several points are still worth noting. First, the description of the magnitude of shear stress is physiologically generable. What is more, the relatively high shear stress is likely to damage cartilage. Therefore, for rehabilitation treatments, a certain kind of limb restraints is needed. Second, it should be noticed that both the endothelial cell and myocardial cell have something to do with angiogenesis, so whether mechanical stress induction of HIF-1α only exists in these type of cells needs further study.

3. HIF-1α regulated by biochemical factors

3.1. HIF activation by pH-dependent nucleolar sequestration of VHL

Recent study done by Mekhail K et al. has revealed that a pH-dependent mechanism will activate HIF by the nucleolar sequestration of VHL (von Hippel-Lindau) as it enables HIF-1α to evade from destruction while its target gene is activated at the same time.

The tumor suppressor protein VHL degrades HIF when it recognizes an E3 ubiquitin ligase's recognition motif in the presence of oxygen [11]. Hypoxia represses above pathway and as a consequence, the target genes of HIF are activated. Researchers tested biochemical attributes and sub-cellular distribution of VHL to clarify the mechanism. First, during normoxia, metabolically active cells will generating extracellular acidification and furtherly causes VHL to be redistributed to sub-nuclear foci, and during acidosis, VHL is redistributed to the nucleolus. After that VHL is demonstrated to be completely confined in the nucleolar as immunofluorescencce microscopy

showed the co-localization of VHL and B23 under acidosis. After it was established through the reversibility of the aforementioned occurrences that VHL is required for the oxygen-dependent degradation of HIF, its functional impact on VHL and HIF due to hypoxia-induced acidosis was subsequently investigated. The conclusion is that when the extracellular pH reached subacid levels, in which VHL was shown in nucleoli, HIF was resistant to destruction following reoxygenation. Additional research has confirmed that nucleolar sequestration of VHL is necessary to stop HIF deterioration after hypoxic-acidotic cells are reoxygenated.

Normally, acidosis is a physiological consequence of hypoxia, and HIF is a series of genes whose protein products will increase cell's adaptability to hypoxia. Based on this finding, other philosophical consequences of hypoxia also activate HIF by certain ways can be hypothesized. At the same time, this finding also provides some clinical significance on cartilage repairing. For example, cellular acidosis is caused by the enhancement of glycolysis physiologically, a kind of targeting glycolysis enhancer can be used to treat corresponding diseases. Next, researchers should pay attention to the way of creating a stable slightly acidic environment locally in vivo without damaging the normal function of the body or vitro stable culture of MSCs under specific subacid conditions.

4. Conclusion

This article mainly talks about the mechanism of three applicational potential non-canonical regulations of HIF-1α, based on which this paper discusses several accessible bioengineering therapies for cartilage repairing. Along with the development of medical science, the standard therapy methods of cartilage damage have been refining and refining again. Thanks to recent development of molecular biology and stem cell biology, therapy can reach the stage of cell rather than tissue. What mentioned in this paper is just several typical and distinguishing non canonical regulations of HIF factors and in fact there are still other pathways that have not been mentioned and their regulatory mechanism may also provide some inspiration of new cartilage damage therapy. Nowadays, these techniques are not yet mature and almost all of them are at the stage of theory, animal experiments or phase I clinical trials. However, its convenience, safety on transplant and universality for all type of patients, MSC induced cartilage repairing will definitely be a heat of future research.

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