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Recent Methods to Facilitate Articular Cartilage Regeneration by Targeting HIF-1α

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Abstract:

Articular cartilage is known to have meager capacity of regeneration, and how to effectively promote repair of it has therefore been a complex issue. Hypoxia inducible factor 1 alpha (HIF-1α), a transcription factor observed to participate in diverse physiological processes, also has been found to play roles in chondrocyte differentiation as well as cartilage homeostasis. This makes manipulation of HIF-1α a potential approach to promote cartilage regeneration and has been widely explored. This review highlights several recent approaches to modulate HIF-1α expression to improve articular cartilage condition: Hydrogel artificial extracellular matrices loaded with HIF-1α-promoting reagents effectively upregulate chondrogenic markers and stabilized cartilage tissue; the combination of PLGA microspheres loading HIF-1α-promoting reagents and PLLA nanofibrous scaffolds yielded similar effectiveness. microRNA-17 was found to restrict the overexpression of HIF-1α and thereby help maintain cartilage homeostasis. Casticin, a natural compound extracted from herbal medicine, has been reported to inhibit inflammatory factors, as well as HIF-1α, and improve the survival of chondrocytes. This review aims to provide comprehensive knowledge of the recent research on HIF-1α target cartilage regeneration, and purpose novel suggestions and insights for the future development of the field.

Keywords: Articular cartilage; HIF-1α; stem cells; biomaterials.

1. Introduction

Articular cartilage (AC) are layers of connective tissue between diarthrodial joints [1] AC could be damaged by sudden mechanical load or prolonged wear and tear under harsh biomechanical stress, which are commonly found in elderly citizens and high-performance athletes [2, 3]: Study of 82 cases in Leiden in the Netherlands showed that only 13% of 90-year-olds had no radiographic symptoms of osteoarthritis [4] Defects in AC could lead to pain and decreased mobility, and have potential to develop into joint degeneration conditions such as osteoarthritis [5].

AC is composed of chondrocytes and extracellular matrix (ECM) produced and maintained by chondrocytes, with extremely limited capacity to regenerate [1, 6]. Chondrocytes can repair ECM which AC is composed of [1]. However, they had limited numbers and their migration was obstructed by dense ECM, thus the ability of chondrocyte to repair cartilage is also limited [7]. Since there are no lymphatics or blood vessels in AC, inflammation that initiated many healing processes would not occur, and it's difficult for other regenerative factors in plasma to be delivered to the injured site without blood vessels [1, 8].

Traditional treatments for AC damage and its associated joint degeneration include usage of braces to reduce the load exerted on the injured site, or anti-inflammatory drugs to relieve the inflammation and pain caused by osteoarthritis. These treatments can moderate the reduction in quality of life caused by AC damage related conditions, but they cannot promote the regeneration of cartilage tissue [8]. To promote the regeneration of articular cartilage, a widely studied method is to mediate the expression of certain growth or transcription factors through physical or biochemical means, thereby ultimately promoting the proliferation and differentiation of chondrocytes [9].

HIF-1α, a long-studied transcription factor, has been regarded as a potential target for articular cartilage repair therapy due to its complex correlation with cartilage regeneration [10]. Some of the recent approaches to manipulate HIF-1α expression to enhance cartilage repair include: artificial extracellular matrix made from alginate hydrogels or PLLA nanofibrous scaffold load with HIF-1α promoting factors [11, 12]; induction of microRNA-17 to restricts HIF-1α signaling [13]; natural compound Casticin to facilitate PI3K/AKT/HIF-1α pathway [14].

The aim of this review is to summarize HIF-1α targeting approaches above, analyze the significance of their result, and propose novel interpretation and strategy for future study of articular cartilage regeneration.

2. Tissue Engineering by Artificial Extracellular Matrix

One strategy for cartilage regeneration is to apply an implant combining extracellular matrix mimicking artificial scaffolds, transplanted stem cells, and biochemical factors to create an optimal microenvironment for cartilage development and stabilization [15]. The following two recent tissue engineering strategies for cartilage regeneration both follow this logic, and use mesenchymal stem cells (MSCs) and HIF-1α targeting biochemical factors. Their most significant difference is the delivery platform used, which is the artificial ECM that biochemical factors are loaded on [11, 12].

2.1 RGD-hydrogel

Hydrogels, due to their properties including biocompatibility and porous structure, are one of the most commonly utilized and studied materials for tissue engineering, drug delivery, and cell culture for medical purposes. In the study by Sathy and colleagues [11], the strategy they adopted was to use alginate hydrogel coupled with RGD peptides and loaded with growth factors and dimethyloxalylglycine (DMOG), as an artificial extracellular matrix in combination with MSCs. DMOG is known for being able to mimic hypoxia and induce HIF-1α expression [16]. RGD peptides induce cell adhesion and have been shown to be effective in promoting cell attachment to different materials, addition of RGD further improve biocompatibility of artificial ECM [17].

In both cell culture of MSCs in vitro and subcutaneous implantation of artificial ECM in vivo, MSCs load with DMOG express HIF-1α in significant higher levels as expected. This effect is more significant under hypoxia conditions, which could be explained by the fact that lower oxygen tension provides more favorable physiological conditions for tissues like articular cartilage.

In culture with presence of TGF-β3, a growth factor that plays key roles in bone tissue development, expression levels of chondrogenic genes such as type-II collagen (Col-2) in MSCs treated with DMOG-RGD-hydrogel were enhanced, while the hypertrophic marker matrix MMP-13 was suppressed. In the presence of optimal concentrations of TGF-β3 , which are shown to promote endochondral bone formation, an increase in chondrogenic markers such as Sox-9 and type I and type II collagen were observed in the DMOG group. This demonstrates that DMOG delivered through the hydrogel artificial ECM effectively increases chondrogenesis and stabilizes cartilage tissue by inhibiting hypertrophy. Smad 2/3 signaling levels were also observed to increase in presence of DMOG. Smad 2/3 signaling is known to inhibit hypertrophy by regulating Sox-9 and plays a role in chondrogenesis and osteogenesis of MCSs, but its relationship with DMOG application remains to be further studied. A worrisome fact is that, in presence of DMOG, elevated expression levels of type X collagen (Col-10), a potential marker of hypertrophy, were observed. However, the increased expression in Col-10 could also be explained by early chondrogenesis. Further investigation might be required to determine which is the critical cause.

Remarkably, the researchers observed enhanced accumulation of cartilage matrix and inhibition of calcification in subcutaneous implants RGD-hydrogel ECM loaded with DMOG. Though the long-term effect of DMOG on MSCs differentiation still needs further study, the observed results are enough to reveal the promising prospects of this strategy in promoting and stabilizing cartilage tissue.

2.2 PLGA microspheres in PLLA nanofibrous scaffolds

Instead of hydrogel, Li and colleagues use alternative biomimetic scaffolds, poly l-lactic acid (PLLA) scaffolds incorporated with poly lactic-co-glycolic acid (PLGA) microspheres. Microspheres made up by lactic acid and glycolic acid synthesized polymer PLGA, were commonly used as therapeutics delivery platform, for their biocompatibility and controllable degradation rate [12]. However, the tendency of agglomeration of PLGA prevents it from working properly in specific tissues. Properties such as plasticity, high porosity and surface area make the microporous-rich nanofibrous scaffold made of PLLA an ideal artificial extracellular matrix. However, loading drugs directly onto the nanofibrous scaffold ECM may cause the drug to be released too rapidly, causing the ECM to lose biological activity in the long-term [18]. The approach they adopted was to apply drug-loaded PLGA microspheres onto PLLA nanofibrous scaffold to obtain an artificial ECM that can release drugs under controllable rate, and then inoculate the scaffold with MSCs.

In addition to dimethyloxalylglycine (DMOG), they also experiment the effect of another HIF-1α facilitating factor, parathyroid hormone-related protein (PTHrP), alone and combine with DMOG. Studies show roles played by PTHrP in regulation of chondrocyte differentiation are critical to maintaining cartilage homeostasis and suppress-

ing hypertrophy, and they were also reported to associate with HIF-1α expression in tumor cells.

In vivo and in vitro experimental results show that scaffolds loaded with DMOG and PTHrP individually increase the expression of HIF-1α and chondrogenic genes such as Sox-9 and type II collagen in MSCs, and the effect is most significant when the two are loaded together. In the in vivo test of subcutaneous implantation into nude mice, most well-developed cartilage tissue was observed in the DMOG&PTHrP combined group.

When DMOG & PTHrP was present in scaffolds, the expression level of phosphorylated YAP (P-YAP) in MSCs was observed to be reduced. P-YAP is the inactivated form of yes-associated protein (YAP), a transcriptional coactivator reported to be involved in the maintenance of chondrogenesis [19]. When HIF-1α inhibitors were applied, a decrease in expression levels of active YAP was observed, suggesting that YAP activation and reduced P-YAP expression are induced by HIF-1α. However, the specific biochemical pathways among HIF-1α, YAP and hypoxic chondrogenic differentiation of MSC still requires further exploration, to understand how their effects are achieved at the molecular level.

Drug release kinetics by tracking cumulative release of PLGA-PLLA nanofiber scaffolds showed that an initial burst release of drugs occurred on the first day, followed by slow release stage. How to adjust attribute of PLGA microsphere such as LA/GA ratio and the PLLA nanofibrous scaffold in which microsphere is located, to control the spatial and temporal pattern of therapeutic delivery according to specific needs, thereby maximize the promotion of cartilage regeneration, still requires further exploration.

Although the specific biochemical molecular mechanism still needs further study, the combined application of DMOG and PTHrP through the combination of PLGA microspheres and PLLA nanofibrous scaffolds has shown good results and prospects in the term of cartilage regeneration.

3. MicroRNA-17

MiR-17, a microRNA belonging to miR-17~92 cluster, was reported to be associated with skeleton growth [20]. According to a study by Zhang and colleagues and previous reports [13], in medial meniscus (DMM)-induced OA mice, the presence of miR-17 in cells was significantly reduced. Zhang and colleagues also found that silencing of miR-17 by antagonist antagomir-17 led to the worsening of OA, while presence of agomir-17 that mimicked miR-17 led to the relative improvement of OA. In mice with miR-17~92 cluster knockout, the severity of OA after DMM increased significantly and could be rescued by agomir-17 injection.

While HIF-1α plays an important role in the synthesis of the ECM, uncontrolled pathological HIF-1α overexpression under inactivation of prolyl hydroxylase 2 (PHD2), HIF main negative regulator, disrupts other processes maintaining cartilage homeostasis [21]. Examples can be collagen modification or protein synthesis requiring glucose oxidation. These disturbances can cause skeletal dysplasia, and was speculated to be responsible for worsening of OA mentioned.

Further quantitative studies revealed that miR-17~92 cKO mice had a significant increase in HIF-1α protein levels, but a decrease in levels of chondrogenic markers such as sox-9, MMP2 and type II collagen. Levels of sox-9 and MMP2 were rescued by agomir-17 injection, which turned down the HIF-1α levels. In group overexpressed miR-17, decrease in HIF-1α level and increase in sox-9 and MMP2 levels were observed. These observations suggest that miR-17 might target and restrict the level of HIF-1α to maintain cartilage homeostasis. Silence of miR-17 would lead to pathological level of HIF-1α expression, which could lead to disruption of cartilage homeostasis and other relative conditions including deterioration of OA.

This study reveals the potential of miR-17 as a target for manipulating HIF-1α to interfere with articular cartilage conditions, and reiterates the importance of appropriate amounts of HIF-1α expression for cartilage homeostasis. Whether it's possible to upregulate HIF-1α expression in controllable and appropriate levels by miR-17 silencing to promote the regeneration of articular cartilage under certain specific conditions, may also be a direction worth exploring.

4. Natural Compounds Casticin

Casticin (CAS), a bioflavonoids phytochemicals synthesized naturally by various plants of the genus Vitex, was reported to inhibit inflammatory cytokine IL-1β [22]. Since IL-1β not only plays roles in degradation process of cartilage and chondrocyte viability, but also involve in activation of PI3K/AKT/HIF-1α pathway, CAS was considered to have the potential to interfere or improve conditions of OA caused mainly by cartilage damage [14].

Liu and colleagues apply CAS on rats with anterior cruciate ligament transection (ACLT)-induced OA, to study the effect of CAS on OA condition and expression levels of signal factor in PI3K/AKT/HIF-1α pathway [14]. Micro-CT morphological evaluation showed that ACLT rats without CAS had higher severity of OA than those who with. In ACLT rats, the expression levels of OA indica-

tors such as MMP-9 and ADAMTS4 were significantly increased, and the expression levels of ECM composing proteins such as COL2A1 and ACAN were significantly decreased, and these changes were significantly reversed in rats applied with CAS.

In IL-1β induced rat chondrocytes cultured in vitro, CAS can reverse the increase in expression of apoptotic regulators like BAX and proinflammatory factors like TNF-α caused by IL-1β to an extent. By doing that, CAS reduces inflammation and over apoptosis, therefore improving chondrocytes viability. The expression levels of PI3K, AKT and HIF-1α, which were significantly increased by IL-1β, were all observed to decrease due to the application of CAS. This result suggests the presence of CAS inhibit PI3K/AKT/HIF-1α signaling pathway, likely by inhibiting IL-1β directly.

CAS has shown the potential to improve OA in animal models in vivo and to increase chondrocyte survival under inflammatory conditions in vitro. However, although CAS can improve chondrocyte survival while inhibiting HIF-1α expression levels, there is no evidence to support the correlation between the two results. HIF-1α is known to play roles in both proapoptotic and antiapoptotic pathways [23], and the improvement of cell viability by CAS is considered to be achieved by inhibition of excessive apoptosis. Therefore, the correlation between changes in HIF-1α levels and the effects of CAS is debatable, and more research is needed to draw further conclusions.

5. Conclusion

Overall, there is great promise to improve cartilage regeneration by manipulating HIF -1α expression levels. Whether using traditional RGD-hydrogel or emerging nanofibers combined with PLGA microspheres, delivery of HIF -1α promoting factors through artificial extracellular matrices successfully increased expression levels of chondrogenic factor in mesenchymal stem cell, and enhanced accumulation of cartilage matrix. Study of microRNA-17 reveals disruption of cartilage homeostasis by uncontrolled HIF-1α overexpression, deepens understanding on the complex associations between HIF-1α and cartilage condition, and suggests a potential new target for promoting HIF-1α. CAS has shown potential in improving osteoarthritis, but the correlation between its improvement in chondrocyte viability and inhibition of HIF-1α levels still needs further discussion and investigation. Since HIF -1α plays diverse roles in numerous physiological processes, how to control its expression to achieve the desired effect is a complex task. While current research has demonstrated the potential of the approach in cartilage regeneration, further research, especially exploration of the specific underlying molecular mechanisms, will provide more understanding to tailor these approaches to improve effectiveness.

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