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Review

Hypoxic conditions modulate chondrogenesis through the circadian clock: the role of HIF-1α

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Abstract: Hypoxia-inducible factor-1 (HIF-1) is a heterodimer transcription factor composed of an alpha and a beta subunit. HIF-1 is a master regulator of cellular response to hypoxia by activating transcription of genes that facilitate metabolic adaptation to hypoxia. Since chondrocytes in mature articular cartilage reside in a hypoxic environment, HIF-1α plays an important role in chondrogenesis and in the physiological lifecycle of articular cartilage. Accumulating evidence suggest interactions between the HIF pathways and the circadian clock. Although the circadian clock is an emerging regulator in both developing and mature chondrocytes, how circadian rhythm is established during the early steps of cartilage formation and through what signaling pathways it promotes the healthy chondrocyte phenotype is still not entirely known. This narrative review aims to deliver a concise analysis of the existing understanding of the dynamic interplay between HIF-1α and the molecular clock in chondrocytes, both in states of health and disease, while also incorporating creative interpretations. We explore diverse hypotheses regarding the intricate interactions among these pathways and propose relevant therapeutic strategies for cartilage disorders such as osteoarthritis.

Keywords: chondrogenesis; hypoxia; circadian clock; transcription factor; osteoarthritis; HIF-1

1. Introduction

Osteoarthritis (OA) is the most common form of chronic inflammatory joint disease (arthritis), and a leading cause of musculoskeletal disability worldwide. It affects synovial joints and is characterized by the progressive degeneration of articular cartilage, the tissue that is involved in transmitting mechanical load and providing smooth articulation of bones but has a limited capacity for regeneration [1]. As articular cartilage degenerates, symptoms such as joint pain, swelling, stiffness, and loss of joint movement arise. OA can affect any joint but most commonly impacts the knee, the hip, and the joints of the hand. OA is a heterogeneous disease with multiple etiologies, clinical phenotypes, and molecular endotypes, which necessitates differential targeting approaches, opening pathways for the development of effective disease-modifying OA drugs (DMOADs) [2].

Despite the socio-economic burden posed by OA, typical management is currently palliative and reactive, rather than proactive and preventive [1]. Joint replacement surgery is a clinically relevant procedure for end-stage OA, but it is associated with more serious adverse events compared to non-surgical treatment options [3]. However, there are no surgery-based, material-based, cell-based or drug-based treatment options currently available that could reliably restore the structure and function of hyaline articular cartilage, despite extensive research and recent developments. There is an urgent need for fundamental research in this field to better understand the causes of why articular cartilage regeneration fails [4].

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Cartilage tissue engineering has promising prospects, especially when chondroprogenitor cells (CPC) are implanted to the site of injury [4]. This has resulted in effective filling of the defect in animal [5] and in human pilot studies [6]. However, a better understanding of the chondrogenic pathways is necessary to assist the delivered cells in forming and sustaining neocartilage at a site where the inflammatory microenvironment is not optimal for supporting chondrogenic differentiation.

Chondrogenic pathways are regulated by multiple external and internal factors [7]. Biological rhythms are known to influence cartilage biology and disruptions to this rhythmicity have been reported to be risk factors for OA [8]. The chondrocyte clock is not only a critical regulator of the healthy chondrocyte phenotype in mature chondrocytes [9], but it has also been demonstrated to be one of the drivers of chondrogenic differentiation pathways [10-12]. The chondrocyte clock is entrained by mechanical cues through daily loading/unloading patterns [8,11]. However, the upstream and downstream effectors that modulate chondrocyte homeostasis *via* the molecular clock are incompletely understood.

One such potential regulator is hypoxia-inducible factor-1α (HIF-1α), which is a heterodimer transcription factor composed of HIF-1α and HIF-1β subunits. Activated by hypoxic conditions, HIF-1α is a key regulator of chondrogenesis [13]. There is now evidence that HIF-1α mediated signaling is coupled to circadian clock synchronization in chondrocytes [14], but the specific mechanism(s) behind this crosstalk are not fully understood. Therefore, the purpose of this narrative review is to offer a synoptic analysis of the current knowledge in the field. We discuss different possible hypotheses about the interplay between HIF-1α and the chondrocyte clock, and offer some related therapeutic strategies in cartilage disorders such as OA.

2. The Molecular Clock in Cartilage Development

Every known organism on planet Earth is characterized by rhythmic patterns in its biological activities. Diurnal changes in mammalian behavioral patterns, metabolic actions, and physiological processes show a specific periodicity [15]. These ~24-hour-long cycles are governed by the intrinsic molecular circadian clockwork. The endogenous clock is regulated by various *Zeitgebers* or time cues [16]. The presence and absence of sunlight is the main regulatory factor of the daily biological rhythms [17]. The suprachiasmatic nucleus (SCN) in the hypothalamus is the central light-sensitive pacemaker of the circadian mechanisms [18]. Central timing signals generated by the SCN are transferred to every tissue in the body, bringing forth a synchronization of the peripheral cell-autonomous molecular clocks [19]. The primary oscillator of the mammalian molecular clockwork is the transcriptional/translational feedback loop (TTFL) [20]. The core clock genes of the TTFL are divided into two types of interlinking mechanisms. During the positive feedback loop, the heterodimer of two transcription factors, BMAL1 (aryl hydrocarbon receptor nuclear translocator-like ARNTL/BMAL1) and CLOCK (circadian locomotor output cycle kaput), bind to the E-box sequence in the promoter region of certain downstream genes and stimulate their expression, particularly in the morning [20,21]. These factors include clock genes that are characteristic for the negative feedback loop (period [PER1-2-3], cryptochrome [CRY1-2], REV-ERB [nuclear receptor subfamily 1 group D member 2, NR1D2/REV-ERB]) and a number of clock-controlled genes (CCGs) which direct tissue-specific gene expression processes [22,23]. Later in the day, the large amount of freshly translated PER and CRY proteins inhibit the function of the positive feedback loop by interacting with the BMAL1:CLOCK complex, thus suppressing their own transcriptional activity [24,25].

Molecular circadian clocks exist in nearly every mammalian peripheral organ and tissue, such as the liver, pancreas, or adipose tissue [26,27]. There is evidence that hyaline and articular cartilage are no exceptions, and differentiating and mature chondrocytes also express the key clock-specific transcription factors at the molecular level, both *in vivo* and *in vitro* [8,28,29]. The chondrocyte clockwork is driven by systemic cues because hyaline cartilage is a peripheral tissue and is not directly sensitive to light. Systemic signals

may originate from biochemical, biomechanical or temperature stimuli [9,30]. In a human *in vitro* model for cartilage formation, core clock genes were found to be inactive in undifferentiated embryonic stem cells; however, upon chondrogenic induction, the key regulatory proteins of the TTFL were detected in the differentiating chondrocytes, and an oscillating expression pattern was distinguished after synchronization [31]. In chondrifying micromass cultures established from chondroprogenitor cells isolated from chicken embryonic limb buds, a functional circadian clockwork was identified after applying serum shock as a clock-resetting method. Not only the clock-specific BMAL1, PER2-3 and CRY1-2, but also the cartilage-specific SRY-box transcription factor 9 (SOX9), aggrecan (ACAN) and collagen type II alpha 1 chain (COL2A1) genes were expressed in a rhythmic oscillatory manner. Additionally, this type of synchronization had a stimulatory effect on chondrogenesis [10]. Upon the knockout of BMAL1 in primary chondrocytes, the expression of the hypertrophic chondrocyte-specific matrix metallopeptidase 13 (MMP13) and RUNX family transcription factor 2 (RUNX2) genes was significantly upregulated [32]. Conversely, PER1 was found to be a negative regulator of chondrogenesis. Sox6 and type II collagen protein levels were elevated after PER1 knockdown in chondrogenic ATDC5 cell cultures [33].

Despite the accumulating data, it is still not clearly known how circadian rhythm is established during the early steps of cartilage formation.

3. HIF-1α: A Master Regulator of Cartilage Development

While most cells of the human body require a higher (ranging from approximately 7.5% to 4% depending on the tissue [34]) concentration of oxygen for cellular respiration and energy production (also termed normoxia or 'physoxia'), chondrocytes, the main cell type of cartilage tissue, reside at lower oxygen levels – this is known as hypoxic condition (O_2<6%). The adaption to this special environment initiates during chondrogenesis and is primarily mediated by HIF-1 [35,36]. HIF-1 is one of the best-known transcription factors induced under hypoxic circumstances. The heterodimer HIF-1 possesses two different subunits; HIF-1α is oxygen-sensitive and is therefore stable in hypoxia, while HIF-1β (aryl hydrocarbon receptor nuclear translocator or ARNT) is stable in normoxia [13]. Although they are constitutively expressed, in normoxia, HIF-1α is degraded *via* oxygen-sensitive prolylhydroxylases. During this, the prolylhydroxylase domain-containing proteins (PHDs) and the asparaginyl hydroxylase FIH-1 hydroxylate HIF-1α, which in this way can be targeted by von Hippel-Lindau protein (VHL), part of the E3 ubiquitin ligase complex [37]. This leads to ubiquitination of HIF-1α, making it a target of the 26S proteasome for degradation. In contrast, in hypoxia, PHDs are repressed and therefore cannot hydroxylate HIF-1α, making it stable and able to combine with HIF-1β to form the HIF-1 heterodimer. After being transported into the nucleus, HIF-1 acts as a transcription factor and binds to genes with a hypoxia response element (HRE) in their enhancer and promoter regions [38,39].

In response to hypoxia, HIF-1 triggers metabolic adaptions in the cell including increased expression of glucose transporters and glycolytic enzymes. Genes with various roles in apoptosis, cell proliferation, angiogenesis, and erythropoiesis are also activated by HIF-1α [40-42]. At the same time, HIF-1α has essential roles in numerous physiological and pathological cell processes like tumorigenesis, inflammation, and tissue development including chondrogenesis [36]. In addition to the most well-known factors that affect cartilage development such as TGF-β, Wnt, SOX5, SOX6, and SOX9 [43,44], HIF-1α also has a pivotal role in chondrogenesis, by linking chondrocyte cell cycle with hypoxic conditions [45].

Due to the lack of vasculature, cartilage tissue develops in hypoxia. For chondrocytes to acclimatize to and survive in such a hypoxic environment, the HIF-1 transcription factor is essential. HIF-1 can influence the homeostasis of cartilage tissue by regulating cell metabolism. Phosphoglycerate-kinase 1 (PGK) mRNA levels affect cell death in the growth plate in HIF-1$\alpha^{+f/-}$;colIIcre or HIF-1$\alpha^{+f/+f}$;colIIcre null animals. In wild type animals, PGK is

present throughout the entire growth plate and has a higher expression level in the upper hypertrophic chondrocytes, where the environment is the most hypoxic. In the null animals, the level of the PGK is lower. Moreover, the knock-out animals are smaller than the controls and there was a remarkable shortening of the forelimbs and the hindlimbs. However, vascular endothelial growth factor (VEGF) expression is also dependent on regulation by HIF-1, which may contribute to the changes in the growth plate *via* the vascularization of the tissue [46,47].

HIF-1α is not only involved in the survival of chondrocytes in hypoxia, but also impacts molecules and pathways that influence the cell cycle, thus controlling cell proliferation and differentiation [47,48]. Moreover, HIF-1α also has an impact on extracellular matrix (ECM) synthesis. Among HIF-1α null and wild type chondrocytes, the ability to produce ECM components changes quite differently from normoxic to hypoxic conditions. Not only the mRNA expression levels of ECM components such as aggrecan and type II collagen are significantly higher, but the protein levels of these ECM elements are considerably elevated in the wild type chondrocytes in hypoxic condition. In some cases, however, the same could be observed in normoxic conditions [49].

4. Interplay between HIF-1α and the Molecular Clock

In recent years, an increasing number of studies aimed to identify interactions between the circadian clock and the HIF pathways [20,50,51]. Crosstalk between the circadian clock and hypoxia-regulated pathways was predicted by the CircaDB online database [52]. Hypoxia can upregulate the expression of PER1, PER2 and CLOCK [53,54]; more specifically, the oxygen sensing moiety of HIF-1α regulates gene expression of BMAL1/MOP3 and CLOCK [50,55,56]. Hypoxia-response element (HRE), the DNA locus where the HIF-1α:HIF-1β heterodimers bind to, is present in the promoter region of differentiated embryonic chondrocyte-expressed genes 1 (DEC1) and 2 (DEC2), which inhibit BMAL1 [57,58]. Therefore, hypoxia through HIF-1α can activate the negative regulators of the circadian rhythm (PER1, PER2, DEC1, and DEC2), leading to the suppression of BMAL1:CLOCK heterodimers.

Interaction between these networks is considered not to be unidirectional. The strongest induction of hypoxia target genes was observed during PER2 peak time in the liver, kidney, and heart [59]. The importance of PER2 is further substantiated by mathematical modeling implying that PER2 has the most crucial role for setting the period of the circadian clock [60]. The hypoxia-induced growth factor VEGF is inhibited by PER2 and CRY1, which results in periodical fluctuations in its gene expression [61]. Recently, CRY1, but not CRY2, was found to negatively regulate HIF-1α and HIF-2α *via* specific protein-protein interactions in mouse embryonic fibroblasts (MEF) [62]. Similar results have been reported in mouse muscles: CRY1 and CRY2 suppressed HIF-1α:BMAL1 heterodimers [63]. Conversely, others have observed that PER2 and CRY1 separately enhanced HIF-1α activity by facilitating HIF-1α recruitment to the enhancer region of its downstream genes, without influencing its expression levels in HeLa cells [64]. Differences between cell types (MEFs, myotubes, and cervical cancer cells), and different CRY binding regions on HIF1 can be an explanation behind this. Apparently, the co-regulation of hypoxia and circadian pathways is more complex, due to the similarity of BMAL1 (ARNTL) and HIF-1β (ARNT) protein structures, which can each form heterodimers with HIF-1α. Low levels of BMAL1 in MEFs decreased HIF-1α accumulation in hypoxia [65]. In line with this, co-expression of BMAL1 and HIF-1α increased the expression of HRE genes, as an adaptation to anaerobic metabolism [66,67]. As the previous findings suggested, there is an approximately 30–50% overlap among HIF-1α and BMAL1 target genes [59]. In addition, the BMAL1:HIF-1α heterodimer also stimulates PER2 transcription in C2C12 myoblasts, further reinforcing the connection between the circadian and hypoxia-related networks. Interestingly, in embryonic stem cells, BMAL1:HIF-1α complexes could not be observed [68]. In macrophages, the BMAL1:CLOCK heterodimer induces NRF2 transcription factor expression, which suppresses reactive oxygen species (ROS) and

therefore HIF1-α, and thus dampens the production of the proinflammatory cytokines IL-1β and IL-6 [69-71]. Notably, NRF2 does not appear to be regulated by circadian clock proteins in all tissues, for instance, in the cerebral cortex [72]. Another possible bridge in the intertwined relationship between the molecular clock and hypoxia is the sirtuin protein family, which are NAD^+-dependent deacetylases. The co-factor of SIRT3 is under circadian control, and the absence of SIRT3 in mitochondria increases ROS levels, resulting in HIF1-α stabilization [73]. When HEK293 and MEF cells were exposed to weak-pulsed electromagnetic field, this elevated ROS production due to changes in the expression of CRY [74]. Another interesting link is by casein kinases (CK1 δ/ε, CK2). In hypoxic conditions, they can post-translationally modify BMAL1 [75,76] and HIF-1α [77], which may strengthen the interconnection between circadian and hypoxic regulation.

There is increasing evidence of bidirectional crosstalk between HIF and circadian pathways in different *in vivo* animal models and clinical observations. Daily rhythms of tissue oxygenation were identified in rodents, which synchronizes the molecular clocks in a HIF-1α dependent manner, and modulation of oxygen concentrations accelerates the recovery from jet lag in a mouse model only if HIF-1α expression is intact [78]. Circadian and HIF-1α pathways affect carcinogenesis and tumor progression by immune suppression. Inhibition of the CLOCK–OLFML3–HIF-1α–LGMN–CD162 axis increases immune response in glioblastoma [79]. Obstructive sleep apnea reduces blood oxygen concentration, which activates the HIF pathways, and therefore alters the circadian rhythm [80], which is speculated to result in metabolic diseases and cardiovascular problems [81]. Dysregulated activation of the NF-κB pathway may lead to chronic diseases such as OA [82]. According to recent results, hypoxia increased NF-κB and proinflammatory cytokine activity; however, after CLOCK silencing, hypoxia-induced inflammatory activity was subdued [83]. This provides evidence for an interesting, multi-directional crosstalk between the circadian clock, hypoxia, and the immune system, also highlighting the fact that the timing of the administration of pharmaceuticals (especially for ischemic and hypoxia-related diseases) is crucial to maximize their efficacy [84].

As described above, cartilage is time-sensitive. As a result of aging and chronic inflammation, the autonomous circadian rhythms were dysregulated in mouse cartilage [85-87]. In mouse OA cartilage, down-regulation of HIF-1α and upregulation of PHD2 was observed [14], which is consistent with an earlier study, where hypoxia maintained normal cartilage homeostasis [88]. Disruption of circadian clock protein expression [14] or HIF-1α depletion [89] can lead to degradation of cartilage ECM *via* activation of matrix-metalloproteinase-13 (MMP-13). Additionally, PER2 was found to respond to DMOG (dimethyloxalylglycine, a PHD inhibitor; in other words, a HIF pathway activator) in a HIF-1α-dependent manner. Furthermore, an *in vivo* study described a connection between circadian rhythms and the balance of collagen anabolism and catabolism [90]. The regulation of HIF-1α by BMAL1 was also confirmed in the mouse intervertebral disc; inhibition of BMAL1 led to damaged nucleus pulposus development [91]. In primary chondrocytes, down-regulation of BMAL1 dampened HIF-1α, HIF-2α and VEGF expression, while its up-regulation had the opposite effect [32]. An increased number of apoptotic cells in BMAL1 knocked-out primary chondrocyte cultures was observed, which can be partly explained by the inhibition of HIF-1α and VEGF expression, whereas HIF-1α can regulate pro- and antiapoptotic genes [39,92].

5. Implications of HIF-1α Dysregulation in Cartilage Disorders

As demonstrated above, HIF-1α plays an important role in chondrogenesis and in the physiological lifecycle of articular cartilage. However, HIF-1α related malfunctions from excessive induction to abnormal downstream signaling are widely reported in cartilage-related pathologies. Regarding the limitations of this review, we focus on the two most-documented fields only: OA and chondrosarcoma.

Inflammation is a key element in the pathogenesis of OA. Articular chondrocytes are partially deprived of oxygen as a result of inflamed synovial membranes using more oxygen [93]. Synovial fluid samples from OA patients demonstrated significantly lower oxygen concentrations compared to healthy samples [94]. Comparing normal and OA joints, detectable differences in HIF-1α protein expression levels can be observed irrespective of the location of chondrocytes within the articular cartilage. Hypoxic conditions, catabolic stress and IL-1β are main factors that can increase HIF-1α accumulation in chondrocytes, delaying rapid progression of OA in early stages [42,95]. HIF-1α plays an essential role in increasing the expression of SOX9 transcription factor directly [96]. Another option to elevate SOX9 levels is *via* BMP2. Stimulating osteo- and chondrogenic differentiation by BMP2 was demonstrated in stem cells in a HIF-1α-dependent manner [97]. Through the upregulated SOX9, COL2A1 and ACAN expression is increased, while chondrocyte differentiation is promoted [98]. Simultaneously with the positive regulation of COL2A1 and ACAN, the inhibition of collagen types I and III also occurs in a HIF-1α-dependent manner [99]. Through HIF-1α, chronic hypoxic conditions significantly decrease expressions of ADAMTSs and MMPs [100], specifically affecting MMP-1 and MMP13 expression levels [101]. Despite the chondroprotective effects of HIF-1α, the newly synthesized matrix components are markers of early alterations in OA cartilage. Besides the previously mentioned examples, the delay of OA progression has other HIF-1α-related maneuvers. By the induction of VEGF expression, HIF-1α can maintain ATP production during oxygen-limited circumstances, thus anaerobic glycolysis allows metabolic adaptation for chondrocytes during hypoxia [102]. The anti-catabolic responses related to GLUT1 and PGK1 genes are key targets of HIF-1α in early OA [103]. Further targeted mechanisms by HIF-1α supporting chondroprotection in early OA are pathways involving anti-apoptotic responses and autophagy [39].

Oxygen levels in tumor niches determine disease progression as cellular responses to hypoxia mainly promote neoplastic evolution. In all skeletal tumors, common cellular mechanisms affected by HIF-1α are cell proliferation, tissue vascularization and metastasis formation. Although cartilage-related malignancies provide rich literature regarding hypoxia, the role of HIF-1α is also implicated in enchondromas [104]. Isocitrate dehydrogenase 1 mutations were reported to induce HIF-1α and consequently influence angiogenic properties and tumorigenicity in the JJ012 human chondrosarcoma cell line [105]. Angiogenesis is promoted by VEGF, but HIF-1α is not the only factor to stimulate VEGF expression in chondrosarcomas [106]. Patient-derived higher-grade chondrosarcoma samples demonstrated increased HIF-1α expression levels associated with up-regulation of Bcl-xL. The survival rate of patients was reciprocal with HIF-1α positivity, suggesting prognostic roles of HIF-1α in chondrosarcoma [107]. The potential for HIF-1α to become a prognostic marker was further strengthened by a meta-analysis on bone tumors that revealed significant correlation between HIF-1α overexpression and overall/disease-free survival [108].

6. Therapeutic Approaches Targeting HIF-1α for Cartilage Repair

Repair of articular cartilage, due to its heavily limited regenerative capacity, is to date a major challenge. Traumatic or degenerative damage to this specialized tissue mounts a significant clinical burden on the health care system [109]. Recent research suggests HIF-1α as a promising target for therapeutic interventions aspiring to repair and regenerate cartilage [110].

As described earlier, HIF-1α, a transcription factor responding to hypoxic conditions, plays a pivotal role in cellular adaptation to low oxygen levels [111]. In chondrocytes, the activation of HIF-1α has the potential to control both autophagy and apoptotic processes. Additionally, it can reduce the synthesis of inflammatory cytokines, manage the ECM environment of chondrocytes, and uphold the chondrogenic phenotype. This phenotypic control extends to the regulation of glycolysis and mitochondrial function associated with OA, ultimately leading to the formation of a more compact collagen matrix that delays the

degradation of cartilage. Consequently, targeting HIF-1α presents a promising avenue for potential therapies in OA by modulating both chondrocyte inflammation and metabolism [110]. At the same time, in laboratory studies, it has been demonstrated that VEGF prompts the proliferation of chondrocytes while also triggering the expression of MMP-13 through the induction of HIF-1α [112].

Various therapeutic strategies have emerged to modulate HIF-1α activity for cartilage repair. One such approach involves pharmacological agents such as the hypoxia-mimetic agent cobalt chloride. Cobalt chloride mimics hypoxic conditions *in vitro* by stabilizing HIF-1α. The outcomes of the experiments by Teti *et al*. indicated that cobalt chloride did not impact cell viability. However, the increase in chondrogenic markers such as SOX9, COL2A1, VCAN, and ACAN relied on the specific cellular origin with most result being quite promising [113]. The prolylhydroxylase (PHD) inhibitor DMOG also stabilizes HIF-1α. Hu *et al*. studied the effects of DMOG-increased expression of HIF-1α in a DMM mouse model and found that it could alleviate apoptosis and senescence *via* mediating mitophagy in chondrocytes [114]. The above studies have demonstrated the potential of these agents for cartilage regeneration.

As gene therapy-based approaches are earning more and more attention, enhancing HIF-1α expression by techniques including viral vectors or gene editing tools have a definite appeal. Okada *et al.* already demonstrated that gain-of-function of HIF-1α in primary chondrocyte cultures can suppress catabolic genes such as MMP13 and HIF2A [115].

Platelet lysate is also gaining increased attention due to its various favorable properties for regenerative medicine. In chondrocyte cultures, platelet lysate promoted the elevation and nuclear transport of HIF-1α, and its binding to HRE [116]. This suggests that HIF-1α could be at least partially responsible for the regenerative effects of platelet lysate. Conversely, platelet lysate may become an easily accessible and safe method for HIF-1α activation.

Future targets may include various interacting partners that all influence the stability and therefore the protein levels of HIF-1α [117]. Prolylhydroxylase domain-containing protein 2 (PHD2) utilizes oxygen and α-ketoglutarate as substrates to carry out the hydroxylation of HIF-1α. N-acetylglucosamine transferase (OGT) stabilizes HIF-1α by reducing α-ketoglutarate levels [118]. Recent findings indicate that HAUSP (USP7) acts as a deubiquitinase for HIF-1α [119]. Under hypoxia, HAUSP undergoes K63-linked polyubiquitination by HectH9, enhancing its ability to deubiquitinate HIF-1α and acting as a scaffold for HIF-1α-induced gene transcription [120]. Plasmacytoma variant translocation 1 (PVT1), a long non-coding RNA, exhibits an oncogenic role in various cancers. Lysine acetyltransferase 2A (KAT2A) is a histone acetyltransferase. Studies reveal that lncRNA PVT1 stabilizes HIF-1α through KAT2A [121]. Furthermore, research demonstrates that the STAT3 protein competes with pVHL, binding to HIF-1α, consequently increasing HIF-1α protein levels [122]. GATA binding protein 3 interacts with both full-length and the N-terminal section of HIF-1α (aa 1–401) during hypoxia, inhibiting the ubiquitination of HIF-1α [123].

Nevertheless, challenges persist in translating these findings into clinically viable therapies. Safety concerns, precise control of HIF-1α activity to avoid undesired effects, and long-term efficacy in clinical settings require further investigation. Additionally, optimizing activation methods and understanding the interplay between HIF-1α and other signaling pathways in chondrocytes are crucial for refining these approaches. Regardless, therapeutic strategies targeting HIF-1α hold immense promise for cartilage repair and regeneration.

7. Future Perspectives and Research Directions

The intricate orchestration of molecular pathways underlying cartilage development involves a delicate interplay between HIF-1α and the molecular clock machinery (*Figure 1)* [124,125]. HIF-1α stands as a central mediator in cellular responses to hypoxia, modulating an abundance of chondrogenic genes [13]. Studies have unveiled the significance of

HIF-1α in promoting chondrogenesis by regulating key transcription factors, including SOX9, essential for cartilage-specific gene expression [39]. Furthermore, key molecules in the hedgehog pathway contain an E-box motif in their promoter regions, making them binding targets of the BMAL1:CLOCK heterodimer complex [126], or, hypothetically, even HIF-1α [127]. The hypoxic microenvironment within developing cartilage activates HIF-1α, accentuating its role in steering mesenchymal stem cells towards the chondrogenic lineage. As described above, accumulating evidence sheds light on the intricate relationship between HIF-1α and the molecular clock machinery. Clock genes, such as BMAL1, CLOCK, and PER, exhibit regulatory control over HIF-1α activity, implicating the circadian rhythm in modulating hypoxia-driven chondrogenesis. Even more interestingly, HIF-1α appears to reciprocally influence the expression of clock genes [80], suggesting a bidirectional interaction between HIF-1α signaling and the molecular clock [10-12] in the context of cartilage development.

While current research has elucidated the impact of HIF-1α and the molecular clock on cartilage development, there are numerous compelling avenues for further exploration. Deciphering the exact mechanisms governing the interaction between HIF-1α and specific clock components in chondrogenesis remains a critical area for on-going research. Examining how external stimuli, such as changes in the environment or pathological conditions, influence this intricate molecular network offers the promise of valuable insights into disorders related to cartilage. A comprehensive understanding of the nuanced regulation of chondrogenesis by HIF-1α and the molecular clock has the potential to open new avenues for therapeutic strategies. Targeted interventions that manipulate either HIF-1α or clock gene expression show potential in advancing cartilage regeneration and alleviating degenerative cartilage diseases. Utilizing the capabilities of these molecular regulators may pave the way for innovative approaches to treating cartilage-related pathologies in the future.

The convergence of HIF-1α signaling and the molecular clock in shaping the landscape of developing cartilage unveils a captivating network of molecular interactions. In addition, due to a multi-directional crosstalk between the circadian clock, hypoxia, and the immune system, the timing of therapeutic interventions is crucial to maximize their efficacy.

Still, there are many unaddressed questions in this context, such as the broader context of signaling molecules regulated by these pathways, the multi-directional interconnections including immune response and inflammatory moieties, and finally to get solid evidence these molecular changes in circadian rhythm and hypoxia response are causes or consequences in cartilage development and cartilage diseases such as OA. Further exploration of these intricate interplays not only deepens our understanding of cartilage biology but also holds transformative potential in advancing therapeutic interventions for cartilage-related disorders.

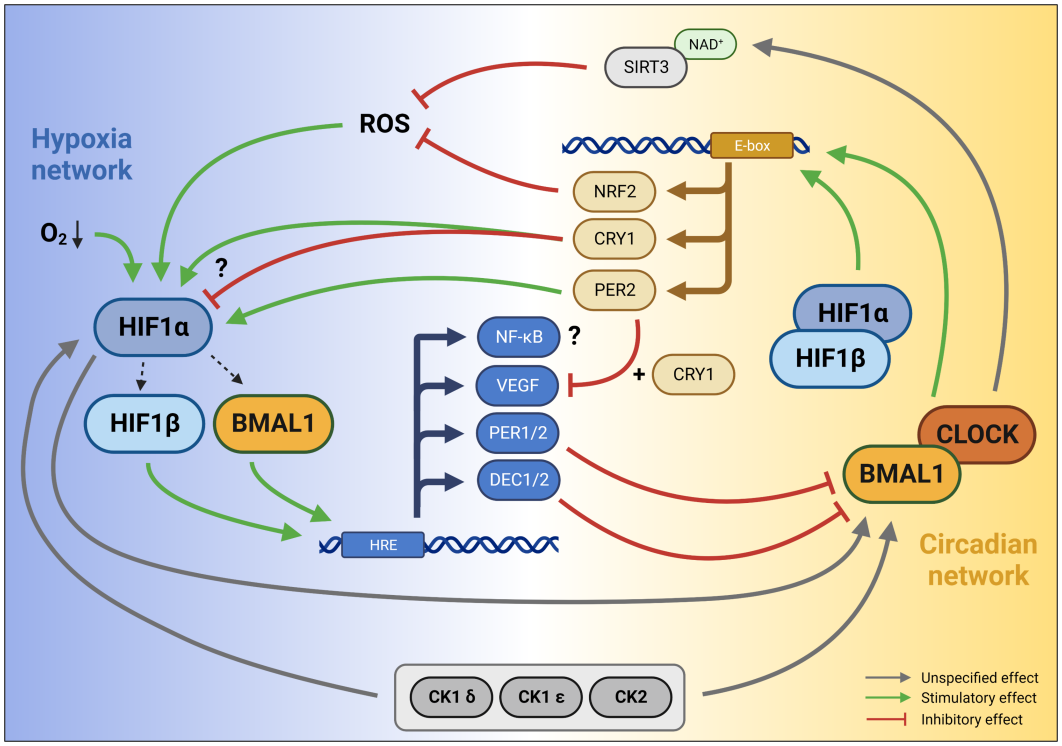

Figure 1. Integration of Hypoxia Signaling and the Molecular Circadian Clock. This schematic illustration highlights the identified interaction sites between hypoxia signaling pathways and the molecular circadian clock in a hypothetical developing chondrocyte. The intertwining of these regulatory networks suggests a complex interplay, influencing various cellular processes and contributing to the coordination of responses to both hypoxia and circadian cues. Please note that the list of pathways shown in the figure is not exhaustive. See abbreviations in text. Created with BioRender.com.

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Abbreviations: CCG, clock-controlled gene; CPC, chondroprogenitor cell; DMOADs, disease-modifying osteoarthritis drugs; DMOG, dimethyloxalylglycine; ECM, extracellular matrix; HIF-1α, hypoxia-inducible factor-1α; HRE, hypoxia response element; MMP, matrix metallopeptidase; OA, osteoarthritis; PGK, phosphoglycerate-kinase; ROS, reactive oxygen species; SCN, suprachiasmatic nucleus; TTFL, transcriptional/translational feedback loop; VEGF, vascular endothelial growth factor.

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