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RESEARCH HIGHLIGHT

HIF-2α - a mediator of osteoarthritis?

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Two recent publications in *Nature Medicine* (June 2010) have focused attention on the role of hypoxia inducible factor HIF-2α in cartilage [1, 2] . Using similar mouse models, both groups provide striking evidence implicating HIF-2α in experimentally induced osteoarthritis (OA). While undoubtedly representing significant advances in the field, care must be taken in interpreting these results, in particular in extrapolating the findings to humans.

Articular cartilage allows friction-free movement and acts as a shock-absorber in the joint. For this reason the tissue cannot afford a delicate blood supply or innervation. At least in larger animals (including humans) a consequence of this lack of vasculature is that articular cartilage is maintained and functions throughout life in a hypoxic environment. This has naturally led to the study of hypoxia and the hypoxia inducible transcription factors (HIFs) in cartilage biology and pathology. HIFs mediate the response of all cells to hypoxia and the protein is continuously produced, but in the presence of sufficient oxygen it is rapidly degraded due to hydroxylation of specific proline residues that target the HIF-α subunit for von Hippel-Lindau tumor suppressor protein (pVHL)-mediated proteosomal degradation. However, when oxygen levels drop below a certain level (typically < 5%), hydroxylation becomes progressively inhibited since molecular oxygen is consumed in this reaction; HIF-α is therefore not degraded and dimerizes with the constitutively expressed HIF-β subunit. The HIF-α/β heterodimer translocates to the nucleus, binding specific hypoxia response element (HRE) consensus sequences (RCGTG) in target genes thus activating their transcription [3]. There are two main HIF-α isoforms (HIF-1α and HIF-2α) and although it is thought they can target different genes, both bind HREs, particularly in *in vitro* assays.

In the mouse, HIF-1α has been extensively studied in growth plate cartilage. Tissue-specific HIF-1α deletion studies have shown that this isoform is necessary for chondrocyte survival and growth arrest [4]. Deletion of pVHL in developing cartilage increases matrix deposition by chondrocytes during growth plate development [5]. Although the authors only considered HIF-1α, pVHL deletion would also be expected to enhance HIF-2α levels. The role of HIFs specifically in the permanent articular cartilage of mice has been less well studied. It is therefore very timely and important that Yang *et al.* and Saito *et al.* have both focused on HIF-2α not only in developing cartilage, but in the age-related disease of osteoarthritis. Surprisingly, both groups found that genetic deletion of one HIF-2α allele (*Epas1*$^{+/-}$ mice) lessened the severity of experimentally induced osteoarthritis [1, 2]. Yang and colleagues also observed huge joint tissue destruction following local injection of Ad-*Epas1* virus into mouse knees. Furthermore, they also investigated mice overexpressing *Epas1* in a tissue-specific manner, and reported spontaneous cartilage destruction in aged mice. It is to their credit that the Korean group have pursued these different, yet complementary approaches to study the function of HIF-2α in murine cartilage. However, one of the reasons why HIFs (or any genes) have not been thoroughly investigated in maintenance of mature articular cartilage in genetic investigations in mice is due to technical difficulties in maintaining adequate tissue-specific (i.e., *Col2a1*-driven) transgene or Cre recombinase expression. The reason for this is that *Col2a1* expression falls significantly in murine cartilage by 4 weeks of age. A major concern, for example with the *Col2a1*-driven *Epas1* transgenic mice developed by the Yang group is whether there is any overexpression specifically in older mice. This is particularly relevant since significant effects are not observed until 45 weeks when we would expect transgene expression to the minimal, if not absent. The authors did show increased HIF-2α levels in the cartilage of transgenic mice, but only at a shorter, unspecified time-point (when there was no degradative changes observable in the tissue).

Epas1$^{+/-}$ mice obtained from the same source were used in the Yang *et al.* and Saito *et al.* studies and these

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mice were clearly shown to have increased resistance to experimentally induced OA [1, 2]. The mechanism for this remains obscure. It is regretful that HIF-1α levels in cartilage were not assessed in these studies. A crucial aspect is whether there is any compensatory increase in HIF-1α due to reduced HIF-2α levels in *Epas1*$^{+/-}$ mice. Although Saito and colleagues imply that this is not the case, they only measured HIF-1α at the mRNA level in *in vitro* cell lines where HIF-2α levels were altered experimentally. Since, as mentioned above, HIF-α is predominantly regulated post-translationally, it is critical to measure steady-state protein levels in the cartilage of *Epas1*$^{+/-}$ mice, rather than rely on mRNA measurements in cell lines.

The two recent reports propose HIF-2α as a catabolic factor in cartilage [1, 2]. However, while Yang *et al*. claim *ADAMTS4* as a direct HIF-2α target, Saito and colleagues report the opposite. However, both groups claim that *MMP13* is a direct target. It must be pointed out, however, that before any potential target can be claimed to be directly regulated, it must be proved to be so under endogenous conditions, for example through chromatin immunoprecitiation (ChIP) assays in primary chondrocytes – such experiments remain to be performed. Interestingly, Rankin *et al.* [6] showed endogenous binding of HIF-2α, but not HIF-1α to erythropoietin (*EPO*) by ChIP in agreement with results from their conditional knockout mice. In contrast, they found that HIF-1α preferentially binds isolated *EPO* HRE fragments *in vitro*. Presumably endogenous activation of *EPO* by HIF-2α requires additional co-factors about which a simple HRE binding assay will not inform.

Although only catabolic target genes were considered in these studies, it is worth pointing out that Saito and coworkers in fact detected significantly increased levels of the key articular cartilage matrix genes, *Col2a1* and aggrecan (*Agc1*) in response to HIF-2α overexpression in primary mouse chondrocytes. Although the authors did not detect any difference in the expression of these anabolic genes in *Epas1*$^{+/-}$ chondrocyte cultures compared to wild-type cells, one would not necessarily expect that there is any difference in HIF-2α protein levels given that these cell culture experiments were performed in normoxia, where HIF will be rapidly targeted for degradation due to hydroxylation of its proline residues. Unfortunately, HIF-2α protein levels were not measured in these experiments.

Both studies also report increased HIF-2α levels in experimentally induced OA cartilage in the mouse and in OA cartilage in humans, but again do not consider HIF-1α. However, placing these results in context of the published literature it must be stressed that other studies have detected elevated levels of HIF-1α in OA cartilage/chondrocytes [7], while HIF-2α was actually reported to be decreased [8]. However, all these findings must also be placed in context of the fact that HIF-2α (and HIF-1α) has a half-life of approximately 5 min in normoxia [9], and therefore the time taken to initially process and fix the tissue samples is critical and can greatly affect the results.

In stark contrast to the two recent HIF-2α studies, induction of OA in BALB/c mice was observed after intra-articular injection of 2-methoxyoestradiol (2ME2) which decreases HIF levels [10]. The authors proposed that the lack of inflammation and inflammatory cytokine induction observed in the study implies that cartilage destruction by 2ME2 does not depend on inflammatory responses, but on a direct chondrocyte response. In contrast, the DMM OA mouse model used by the Korean and Japanese groups in the HIF-2α studies [1, 2] is known to have a strong inflammatory component, while collagenase injection and Ad-*Epas1* injection used by Yang *et al*. actually causes clear synovitis [1]. Such strong inflammatory responses are not normally thought to occur in the chronic condition of OA in humans. However, use of compounds such as 2ME2 is also far from optimal as they are known to be non-specific, a drawback that is avoided by the use of genetically modified mice.

Finally, a critical aspect to consider with respect to therapeutic application is: do these findings in mice reflect the human condition? First, we must consider the physiochemical conditions of the tissue in each species – both are avascular, but while murine articular cartilage in the knee is only a few cells across, in humans it is a few millimetres thick! The implications for oxygen diffusion are obvious. Furthermore, the mechanical forces experienced in the joint are hugely different between man and mouse [11]. Interestingly, hypoxic induction of cartilage matrix genes is very weak in murine chondrocytes compared to that observed in chondrocytes obtained from larger animals (cows and humans) [12, 13]. It may be that mechanisms have evolved specifically in larger animals which enable the articular cartilage to use hypoxia to promote its tissue-specific function, i.e., production of a load-bearing matrix. In extensive studies using normal human articular chondrocytes we have previously shown that HIF-2α (but not HIF-1α) is responsible for hypoxic induction of cartilage matrix genes [13, 14]. We showed that for most cartilage matrix genes this occurs, not by direct hypoxic induction of the genes but through key cartilage-specific transcription factor SOX9 [14]. This strongly suggests that HIF-2α is likely to be an anabolic factor in human articular cartilage, which contrasts with the recent findings in mice [1, 2]. Yang *et al.* found that HIF-2α was induced in mouse articular chondrocytes by treatment with the catabolic cytokine IL-1β (they did not investigate HIF-1α responses) [1]. In direct contrast, however, we have observed that HIF-2α protein levels are not affected (or are slightly decreased) by IL-1β treatment

of human articular chondrocytes (unpublished observations), while HIF-1α protein is strongly increased (in agreement with previous reports [15]). Saito and colleagues claim that HIF-2α is acting independently of oxygen-dependent hydroxylation in murine chondrocytes [2]. In contrast, in human articular chondrocytes, HIF-2α is strongly regulated by prolyl hydroxylation, and can be stabilized even in the presence of atmospheric oxygen by specific inhibition of the HIF-targeting prolyl hydroxylases (PHDs) [9]. Furthermore, such PHD inhibition in human chondrocytes enhances expression of the main cartilage matrix genes [9]. Taken together, this suggests that there can be fundamental differences in the way human and murine chondrocytes respond to the same signal, in particular if that signal is hypoxia-related. Therefore, while genetic studies in the mouse remain an invaluable aid to our understanding of cartilage pathophysiology, if we are to develop successful therapeutic interventions in OA, we must be ever mindful of fundamental species differences.

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