The Role of the Hypoxia-inducible Factor (HIF) Pathway in Normal and Osteoarthritic Meniscus.

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Introduction: Meniscus injury and the hypoxia-inducible factor (HIF) pathway have been independently linked to osteoarthritis (OA) pathogenesis in cartilage1,2, but the HIF pathway has not been explored in the meniscus. Pro-inflammatory stimulation of meniscus cells is known to increase catabolic activity and matrix degrading protein expression3. Increased catabolic activity in meniscus cells is also associated with the NF-κB pathway3, which has been shown to interact with the HIF-2α pathway in cartilage1, 2.

The purpose of this study was to identify and evaluate the response of the HIF pathway in normal and OA meniscus and to examine the effects of Epas1 (HIF-2α) insufficiency in mice on early OA development. We hypothesized that increased HIF-2α but not HIF-1α activity would lead to increased catabolism in human meniscus cells and knockdown of Epas1(HIF-2α gene) would be protective for OA in a mouse surgical model.

Methods: Normal (n=16) and OA (n=31) human meniscus specimens were obtained with Institutional Review Board protocol approval. Specimens were used for immunohistochemical evaluation and cell culture studies.. Meniscus cells were treated with proinflammatory stimulation, including interleukins(IL)-1β, IL-6, transforming growth factor(TGF)-α and fibronectin fragments (FnF), or with the NF-κB pathway inhibitor hypoestoxide (HE).HIF and HIF target gene expression were analyzed at 6 and 24 hours. Gene expression was also evaluated with HIF-1α and HIF-2α (Epas1) overexpression and knockdown in meniscus cells. Wild-type (n=36) and Epas1+/- (n=30) heterozygous mice underwent the destabilized medial meniscus (DMM) surgery with Animal Care and Use Committee approval. Mice were evaluated at two and four weeks post-operatively for early OA development using a previously published histologic grading system4. . Statistical analysis was performed with multivariate general linear models with appropriate post hoc testing when indicated. Correlations were determined using Spearman’s rho. Analysis was completed using SPSS and Prism. For all analyses statistical significance was set at p≤0.05.

Results: HIF-1α and HIF-2α immunostaining and basal HIF gene expression (p=0.41) did not significantly differ in normal and OA meniscus specimens. Pro-inflammatory stimulation did not significantly alter HIF-1α and Epas1 RNA levels (respectively, p=0.43, p=0.37) while these same stimuli significantly increased meniscal cell MMP-1 and -3 gene expression (MMP1: IL-1β, p=0.002; IL-6, p=0.005; FnF, p=0.002. MMP3: IL-1β, p=0.003; FnF, p=0.001; Figure 1). TGFα increased Col2α1 expression (p=0.010) and FnF increased Sox9 expression (p<0.001). Following pro-inflammatory stimulation, HIF-1α and Epas1 gene expression positively correlated with MMP1 (R=0.403, p<0.001; R=0.408, p<0.001) and MMP3
expression (R= 0.641, p<0.001; R= 0.526, p<0.001). Both HIF-1α and Epas1 positively correlated with Sox9 expression (R=0.515, p<0.001; R=0.700, p<0.001) and the Sox9 target, collagen type II (R=0.472, p=0.003; R=0.501, p=0.002). Epas1 overexpression significantly increased Col2a1 expression. Knockdown of HIF-1α and Epas1 did not significantly alter target gene expression. Treatment with HE significantly reduced HIF expression in OA meniscus cells (p=0.02) but not normal cells (Figure 2A) and inhibited the increased HIF expression in normal cells treated with pro-inflammatory stimulation known to increase NF-κB activity (Figure 2B).

Both wild-type and Epas1+/- mice developed OA following DMM surgery (Figure 3). There were no significant differences between genotypes at either 2 or 4 weeks after surgery in articular cartilage structure (ACS) score or meniscal area.

**Discussion:** Unlike previous studies in articular cartilage, we did not find evidence that HIF-1α and HIF-2α have differential effects on MMP expression in meniscal cells. Rather, overexpression of either HIF isoform increased expression of both catabolic and anabolic genes. Additionally, Epas1 insufficiency did not protect against early OA development in the mouse at 2 or 4 weeks after DMM surgery. These findings indicate that further work on the HIF pathways in the various joint tissues affected by OA is needed before pursuing HIFs as therapeutic targets for OA, in particular for early disease.

**Significance:** While the HIF pathway may be involved in joint tissue pathology, our findings indicate that further work on the HIF pathways in multiple joint tissues affected by OA is needed before pursuing HIFs as therapeutic targets for OA, in particular for early disease pathogenesis.
Figure 1: Effects of pro-inflammatory stimulation on MMP and anabolic gene expression in normal meniscus. Data are mean ± 95% confidence interval (n≥6 donors).
Figure 2: Effects of hypoestoxide on HIF expression. Data are mean ± 95% confidence interval (n≥6 donors).
Figure 3: Representative histology and analysis following destabilized medial meniscus surgery in mice.