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Purpose: We investigated if an anti-TNF-α TFO, employed as anti-gene strategy, could be an alternative to antisense technology in silencing TNF-α activation during articular cartilage development. In this study, we analyzed in vitro and in vivo anti-inflammatory potentialities of an anti-TNF-α TFO, as judged from effects on two rat arthritis models.

Methods: The inhibitory activity of this TFO on articular cells stimulated by IL-1β (synoviocytes and chondrocytes) was assessed and compared to that of small interfering RNA (siRNA) in vitro, at mRNA expression levels by real-time RT-PCR, as well as at proteins level (TNF-α & NO release). In vivo, the biological effects of a preventive intraarticular injection of such an oligonucleotide were first investigated in an acute arthritis model, by the follow-up of clinical (body weight, pain, joint swelling), biochemical (anabolism loss, cytokines levels) and histological (synovium, cartilage, and bone) parameters. We then confirmed the efficiency of a preventive ia injection of anti-TNF-α TFO in an immunological experimental chronic arthritis model, according to similar parameters (clinical, biochemical and histological assessments).

Results: In vitro, we have demonstrated that a TFO designed to target TNF-α promoter was able to inhibit mRNA expression (86%) and to prevent TNF-α release into supernatants (62%) as well as NO release (80%). The inhibition rate, at mRNA level, was similar to the one observed with TNF-α specific siRNA, the main advantage of TFO being the concentration used (1nM vs 75 nM for siRNA). A difference was observed at proteins level, with a 20% more efficient inhibition with TFO, compared to siRNA.

The use of the anti-TNF-α TFO as a preventive and local treatment in both acute and chronic arthritis models significantly reduced disease development. In the acute inflammatory model, the TFO provided a stronger inhibition than the siRNA, with a long lasting biological effect, confirmed by histological assessment. In the immunological RA model, we have demonstrated that a preventive ia injection of the TFO lead to a significant correction of body weight distribution (incapacitancy test), confirmed by the histological grading of the knees. Indeed, fibrosis and infiltration were significantly decreased compared to arthritic rats. Analysis of synovial fluid has demonstrated a significant decrease of inflammatory mediators like cytokines (TNF-α, IL-1β, IL-2, IL-5) and chemokines (MCP1, MIP-1α) in animals pretreated with TFO compared to naive. Furthermore, the TFO efficiently blocked synovitis and cartilage and bone destruction in the joints.

Conclusions: We showed the effectiveness of siRNA and TFO in modulating both in vitro and in vivo inflammatory processes. Interestingly, silencing was increased with TFO, enabling improved protection of articular components. We extended our findings by demonstrating for the first time that in rats developing arthritis, a preventive injection of anti-TNF-α TFO led to local and systemic TNF-α inhibition associated with improvement of ancillary clinical signs of arthritis. The results presented here provide the first evidence that gene targeting by anti-TNF-α TFO modulates arthritis in vivo, thus providing proof-of-concept that it could be used as therapeutic tool for TNF-α-dependent inflammatory disorders.

462 SUSTAINED REGULATION OF INFLAMMATION IN CHONDROCYTES BY BIOMECHANICAL SIGNALS VIA THE JAK3-STAT3 PATHWAY

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Purpose: Exercise is essential for maintaining cartilage health and therapeutic for degenerating cartilage during the progression of early osteoarthritis (OA) as shown in our animal models. At the molecular level, we have also shown that physiological magnitudes of mechanical forces are anti-inflammatory and suppress the transcriptional activities of nuclear transcription factor kappaB (NF-κB) by interfering NF-κB signaling cascades. In this study, we examined the role of IL-11 via JAK3-STAT3-SOCS3 signaling cascade in inducing sustained anti-inflammatory state by physiological magnitudes of dynamic compressive strain (DCS). These findings further correlate with the attenuation of pro-inflammatory genes during the progression of OA by exercise in an animal model.

Methods: OSU Institutional Animal and Care Committee preapproved all protocols. Rat chondrocytes (5 x 105 cells/scaffold) were inoculated on 3D 6x3 mm cylindrical scaffolds fabricated by electrospinning of poly(ε-caprolactone), 6 days prior to experimentation. Subsequently, the constructs were subjected to various conditions: 1) untreated control, 2) IL-1β (1 ng/ml), 3) DCS alone (10% cyclic strain at 1 Hz), or 4) IL-1β and DCS for various durations. The effects of DCS on the overall gene expression were analyzed by Affymetrix Rat GeneChip 1.0 ST microarrays and the results confirmed by real-time PCR using custom designed primers for IL-11 and SOCS3. Activation of the JAK-STAT pathway was examined via phosphorylation of JAK1,-2, -3, and phosphorylation of STAT3, -5, and IL-11 protein expression by Western blot analysis (WBA). To investigate the relationship between IL-11 and JAK-STAT activation, IL-11 was knocked down by siRNA. All experiments were performed at least in triplicate and statistical significance calculated by ANOVA with Tukey's post-hoc. Results: Microarray gene expression analysis on the ACs subjected to IL-1β (1 ng/ml) alone or 10% DCS in the presence of IL-1β revealed that 1163 genes out of approximately 27000 genes (detectable by GeneChip) were significantly regulated by DCS. These findings by demonstrating for the first time that gene targeting by anti-TNF-α TFO modulates arthritis in vivo, thus providing proof-of-concept that it could be used as therapeutic tool for TNF-α-dependent inflammatory disorders.