REGULATION OF ANABOLIC AND CATABOLIC PATHWAYS BY OSTEOGENIC PROTEIN-1: GENE ARRAY DATA

INTRODUCTION:
Osteogenic Protein-1 (OP-1) or bone morphogenetic protein 7 (BMP 7) is expressed in human adult articular cartilage. The levels of OP-1 expression and synthesis decrease with age and with cartilage degeneration and osteoarthritis (1,2). Recombinant OP-1 (rhOP-1) is a potent growth factor for cartilage repair due to its pro-anabolic and anti-catabolic activities. In earlier studies, inhibition of endogenous OP-1 expression with antisense oligonucleotides caused a significant decrease in aggrecan gene expression, aggrecanase, proteoglycan synthesis, proteoglycan content, and depletion of Saffranin O stain (3). The goal of the current study was to use a gene array approach to evaluate chondrocyte expression of anabolic genes (growth factors with corresponding receptors and regulators of their downstream signaling as well as cartilage matrix proteins) and catabolic genes (proinflammatory cytokines/chemokines, their receptors, proteases with various modes of action and transcription factors) under conditions where OP-1 expression was inhibited by antisense approach or OP-1 signaling was activated by rhOP-1.

METHODS:
Human normal adult articular cartilage was obtained from the ankle joints of 12 tissue donors, age 55-70 y/o, within 24 hours of death through collaboration with the Gift of Hope Organ & Tissue Donor Network (Elmhurst, IL) with Institutional Review Board approval and appropriate consent. Chondrocytes were isolated by enzymatic digestion overnight and plated at high-density in monolayers for 24 hours in the presence of fetal bovine serum for attachment. Cells were either transfected with OP-1 antisense oligonucleotides as described (3) or treated with rhOP-1 (100 ng/ml) for 48 hours followed by RNA isolation. Gene expression profile was analyzed by HG-U133A gene chips from Affimetrix. RNA within each treatment group was pooled from all donors. Statistical analysis consisted of 1) analysis of differentially expressed genes under single experimental condition in comparison to the corresponding control (up- or down-regulated in the presence of OP-1 antisense or rhOP-1); 2) analysis of differentially regulated genes by both treatments (OP-1 antisense and rhOP-1); and 3) gene ontology. Cut off was chosen at 1.5-fold and major gene array data were verified experimentally in vitro or by real-time PCR.

RESULTS:
The results of gene array analysis showed that the inhibition of endogenous OP-1 mRNA expression led to down-regulation of a number of anabolic genes among which were the members of the IGF-1 signaling pathway: IGF-1 receptor (1.73-fold decrease), IGFBP-5 (1.9-fold) and IGFBP-7 (1.58-fold, P<0.01). In vitro validation experiments confirmed and extended these findings: in chondrocytes treated with OP-1 antisense, expression of IGF-1 and its receptor was down-regulated, while stimulation with rhOP-1 up-regulated expression of these genes. This in turn restored the response to IGF-1 that is diminished with aging in human adult articular cartilage. Antisense inhibition of OP-1 modulated expression of members of the TGF-β/BMP signaling pathway. Expression of the following genes was down-regulated: GDF-10 (1.86-fold), activin type 1 and type 2 receptors (2.42-fold), BMP receptor type 1A (or ALK-3; 1.83-fold), TGF-β receptors 1, 2, and 3 (1.5-fold), Id proteins 2-4 (up to 2.32-fold), and binding protein gremlin (1.94-fold). Fewer genes from this pathway were up-regulated under OP-1 antisense treatment, primarily GDF-15, activin A, and follistatin (about 2-fold). Stimulation of chondrocytes with rhOP-1 induced gremlin and GDF-10 genes indicating tight regulation by OP-1 (their expression was inhibited by OP-1 antisense). Surprisingly, rhOP-1 caused an inhibition of BMP-2 (2.67-fold), GDF-15 (3.04-fold), and activin (2.32-fold) mRNA expression. Real-time PCR confirmed the decrease in BMP-2 gene expression after treatment with OP-1. As expected, certain matrix protein genes were regulated by OP-1: rhOP-1 induced collagen type IX, COMP, and cadherin genes; while OP-1 antisense reduced collagen type VI, versican, syndecan, bone sialoprotein, cadherin, and osteonectin. Among catabolic genes, the most affected by OP-1 were leukemia inhibitory factor (LIF), IL-6, IL-8, IL-11, IL-1 and IL-6 receptors, NF-κB and AP-2. Inhibition of OP-1 by antisense oligonucleotides caused a 2-fold increase in their expression (p<0.001), while treatment with rhOP-1 dramatically inhibited these genes: LIF by 15.86-fold; IL-6 by 2-fold; IL-8 by 4.01-fold; and IL-11 by 8.69-fold (p<0.001). Real-time PCR experiments confirmed these findings. Noteworthy, OP-1 was not only able to reduce expression of autocrine cytokine genes, but also co-protected the inhibitor with IL-1β and IL-6 on proteoglycan synthesis in different culture systems. In addition, rhOP-1 inhibited MMP-14 and TIMP-3, while the expression of MMP-2, -9 and TIMP-4 was decreased after OP-1 antisense treatment, which also caused a decrease in expression of ADAM-7 and 10, ADAM-Ts, and cathepsins B, C, and S. With regard to apoptosis-related genes, rhOP-1 inhibited caspase 4, programmed cell death gene, and calpain 9; while OP-1 antisense treatment caused a decrease in caspases 2, 6, 8, and 9.

DISCUSSION:
Gene array data provided a powerful overview of the genes and pathways that are regulated by OP-1 in normal human adult articular cartilage. Although this analysis was limited to only one time point (48 hours) and to chondrocytes in monolayer culture, it strongly suggests a critical role for OP-1 in maintaining cartilage homeostasis. Changes of the gene expression profile support previous findings that OP-1 is an inducer of matrix synthesis and is able to overcome or down-regulate degenerative processes stimulated by catabolic mediators. Surprisingly, no effect was detected on the major cartilage matrix components, such as aggrecan and collagen type II. However, we anticipate these genes are regulated by OP-1 at different time points (a subject of a follow-up study). OP-1 regulation of the IGF-1 pathway is consistent with anti-apoptotic properties of OP-1 (4) due to the known survival promoting affect of IGF-1. To our knowledge, this is the first report to show that OP-1 is able to regulate expression of growth factors that belong to the same TGF-β/BMP family, their receptors and downstream signaling molecules. The biggest surprise was the finding of inhibition of the BMP-2 gene by rhOP-1. This suggests a negative feedback loop such that high levels of OP-1 inhibit expression of other BMPs. In conclusion, the results undoubtedly prove that OP-1 regulates numerous metabolic pathways that are not only limited to its anabolic function, but also to its anti-catabolic activity. These findings provide strong justification for the application of OP-1 protein as a therapeutic treatment for cartilage regeneration and repair.

REFERENCES:

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