Differential display reverse transcription PCR reveals IL-1 induced gene expression patterns in human articular chondrocytes

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IL-1 exerts diverse effects on the metabolism of articular chondrocytes, including inhibition of proteoglycan synthesis and stimulation of matrix metalloproteinase synthesis. Therefore it is believed that IL-1 might play an important role in cartilage degradation in osteo- and rheumatoid arthritis. To improve our understanding of IL-1 induced effects on overall gene expression patterns of human articular chondrocytes, we used a novel mRNA fingerprinting technique: Differential Display Reverse Transcription–PCR (DDRT-PCR) (Liang and Pardee 1992). The reported high sensitivity of this powerful technique promised to enable work with human articular cartilage, a tissue from which only small amounts of RNA can be obtained, and to get access to low abundance transcripts, difficult to detect with conventional subtractive hybridization studies. Our goal was to eventually identify new marker genes and pharmacological target molecules for drug intervention in IL-1 mediated cartilage degradation.

Methods

Articular knee cartilage from patients undergoing joint replacement surgery for osteoarthritis was digested with pronase and collagenase; isolated chondrocytes were suspended in alginate beads. The cells were fed daily with Dulbecco's Modified Essential Medium / Ham's F12 medium (50/50) containing 10% fetal bovine serum and 25 µg ascorbic acid/ml. After three days of culture, the beads were divided in two groups and the medium of one group was supplemented with 5 U/ml rhIL-1ß. Both cell populations were cultured under these conditions for further three days. After dissolving the beads, 20 µg total RNA was prepared from 10^7 chondrocytes. RNA was subjected to Differential Display as described by Liang and Pardee 1992 and Bauer et al. 1993. A side by side comparision of the DDRT-PCR band pattern from each cell population created differentially displayed bands for further analysis. Those were cut out and eluted from the gels, reamplified by PCR using appropriate primer combinations and subcloned by TA cloning. Sequences of PCR fragments were compared with sequences in DNA databases. Finally, differential expression of interesting fragments was verified by northern blot analysis using radiolabeled cDNA probes. Intensities of bands were quantified with a phosphorimager.

Results

100 different primer combinations (25 oligodecamer primers, 4 T_{12}MN-primers) generated a total of approximately 10,000 PCR products for each cell population after DDRT-PCR, which should represent 53 percent of all expressed cellular genes according to theoretical calculations (Liang 1994). 123 PCR bands appeared as differentially expressed bands. To validate those PCR products, we compared the first display pattern with a second pattern obtained from another patient. 53 of the original 123 bands were reproducibly displayed; of those 68 percent arose from IL-1ß stimulated chondrocytes. After subcloning and sequencing, three sequences showed a significant sequence homology to known genes. Fragment TAU 1/1 is 100 percent identical to human osteopontin cDNA (Denhard 1993) and fragment TTU 2/2 shows a 99 percent sequence identity to human calnexin cDNA (Bergeron 1993). Both fragments appeared downregulated in stimulated chondrocytes. In contrast, TAU 7/2 is upregulated in stimulated cells and displays 99 percent sequence similarity to human TNF-stimulated gene-6 (TSG-6) (Lee et al. 1992, Wisniewski et al. 1993). A quantitation of northern blots of RNA from chondrocytes isolated from a third patient, probed with labeled calnexin and osteopontin fragments, revealed an expression level of 60 percent for calnexin and 21 percent for osteopontin in IL-1ß stimulated chondrocytes versus unstimulated chondrocytes. Most detected sequences show no significant homology to known genes

Discussion

This is the first direct demonstration of the pleiotropic effects by IL-1 on the overall gene expression of

human articular chondrocytes. DDRT-PCR enables the detection of differentially expressed genes, even when only minute amounts of RNA are available. By comparing the PCR band patterns originating from two different patients, we found reproducibly, differentially displayed bands and those which differ between patients. The latter might reflect different disease stages and await further evaluation in terms of their correlation to specific clinical patterns in OA. Sequencing of differentially displayed fragments already revealed the IL-1 dependent differential expression of three known genes: calnexin, osteopontin and TSG-6. The differential downexpression of calnexin, a molecular chaperone and component of the quality control system of the ER, may reflect secondary changes in basic cellular metabolism elicited by IL-1. The downexpression of osteopontin (Ca^{2+} binding, RGD domain containing integrin ligand) and the upregulation of TSG-6 (HA-binding CD44 homolog) may reflect more immediate cytokine directed events with possible closer relation to the etiopathology of OA. Thus, DDRT-PCR is able to yield interesting candidate genes, potentially involved in the etiopathology of OA. Further studies of those others, differentially displayed fragments of unknown sequences, may lead to the identification of new enzymes, transcription factors, or other genes useful as markers or targets for drug modification in degenerate joint diseases.

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Concentration and size distribution of IGF-I in human normal and osteoarthritic synovial fluid and cartilage

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The role of free insulin-like growth factor (IGF-I) in stimulating proteoglycan (PG) synthesis in cartilage has been well documented (e.g., McQuillan et al., 1986). Equally well documented is the enhancing effect of serum addition to the incubation medium. However, it is by no means clear what, if any, is the connection between the latter two: thus in serum IGF-I is not present in free form but exists in the form of complexes, mainly as a "large complex" of MWt around 140 kD and to a lesser extent as a "small complex" of MWt around 50 kD. Molecules of this size are largely excluded from the matrix of normal human articular cartilage. Moreover, it should be remembered that cartilage in vivo is not in contact with blood, but with synovial fluid (SF). Information about IGF-I in SF is scarce: there is only one set of data available (Schalkwijk et al. 1989) and these only for total IGF-I content.

Thus, the following questions arise: what are the forms and the amounts in which IGF-I is actually present in human cartilage? Is this IGF-I likely to originate from synovial fluid or is it produced locally? Are the amounts of IGF-I actually found comparable to those known to be required for the stimulation of PG synthesis? Is the general picture the same in OA as in normal joints?

Materials and methods

Normal human sera were obtained from the Israeli Blood Bank and normal synovial fluids were taken at postmortem at a Forensic Institute from knee joints of subjects with no history of arthritic diseases. Osteoarthritic synovial fluids were obtained in the course of diagnostic procedures. Normal femoral heads were obtained freshly at operations for femoral neck fractures or at postmortem. Osteoarthritic femoral heads and femoral condyles were obtained at operations for total joint replacement. All operations were performed under sterile conditions and the cartilage (0.5 to 1.5 gm) was incubated in two successive 3 ml aliquots of culture medium (DMEM+0.1% BSA), some samples at 4 °C and some at 37 °C, for three