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.

References

Bauer D, et al. Nucl Acids Res 1993; (21): 4272–80.
Bergeron J, et al. TIBS 1993; (19): 124–8.
Denhardt D T. FASEB J 1993; (7): 1475–82.
Lee T H, et al. J Cell Biol 1992; (116): 545–57.
Liang P. Methods in Mol Genet 1994; (5): 3–16.
Liang P, Pardee A. Science 1992; (257): 967–71.
Wisniewski H G, et al. J Immunol 1993; (151): 6593–601.

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

Rosa Schneiderman[1], Nahum Rosenberg[2], Yehuda Hiss[3], Phillip Lee[4] and Alice Maroudas[1]

[1]Julius Silver Institute of Biomedical Sciences, Department of Biomedical Engineering, Technion – Israel Institute of Technology, Haifa, Israel, [2]Department of Orthopaedics, Rambam Hospital, Haifa, Israel, [3]Leopold Green Institute of Forensic Medicine, Tel-Aviv, Israel, and [4]Diagnostic Research Laboratories, Webster, Texas, U.S.A.
Correspondence: Alice Maroudas, Department of Biomedical Engineering, Technion – Israel Institute of Technology, Haifa 32000, Israel. Tel +972-4-294118. Fax +972-4-234131.

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

days. The incubation media were ultrafiltered through 20 and 100 kDa membranes and the retentates and filtrates analyzed for IGF-I, using an RIA kit (DSL, Texas). We refer to the species passing through the 20 kDa as free IGF-I, those passing through the 100 kDa, but not the 20 kDa filter as small complexes (SC) and the species retained by the 100 kDa as the "large complexes" (LC). Control sera and synovial fluids were subjected to a similar process of ultra-filtration and analysis.

Results

Comparison between human sera and synovial fluids. We found a large difference between normal synovial fluids and sera with regard to both the total IGF-I and the relative proportions of the different species. The total concentration of IGF-I is four to ten times lower in SF than in HS; this difference is mainly due to the dramatic decrease (10 to 20 fold) in the amount of the large complex in SF as compared with HS. Free IGF-I content is extremely low in both HS and SF and there is no significant difference between the two. The small complex accounts for approximately 60% and LC for 40% of the total IGF-I content in SF.

In the case of serum, there is a clear decrease in the total IGF-I content with age, a finding which has been well documented in the literature. This decrease is due to a decrease in both LC and SC. On the other hand, there appears to be no age-related changes in the IGF-I content of synovial fluid.

As regards synovial fluids from osteoarthritic joints. Our preliminary results indicate higher levels of both SC and LC than in the case of normal fluids, but the number of fluids examined is so far too small to be sure of the statistical significance of this finding.

Normal and osteoarthritic human cartilage

The overall level of IGF-I in normal cartilage was found to be very low, especially in the middle and deep zones. In the surface slices it was of the order of 9 ngm per gm tissue, with SC predominating. For the middle and deep zones, the total IGF-I concentration varied between 1 and 3 ngm per gm tissue. LC was either zero or only traces were present, both in the surface and in the middle and deep zones. The concentrations of IGF-I in the second wash were always much lower than in the first wash, at 4 °C as well as 37 °C, implying that no measurable autocrine production was occurring.

The data for OA cartilage are very different. The total IGF-I varied between 30 and 100 ngm per gm of tissue for the surface and between 10 and 40 ngm for the middle and deep zones. The values for free IGF-I and for LC often actually exceed the mean values found in SF. No difference was found between the 37 °C and the 4 °C results either for normal or for OA cartilage, which shows that no autocrine production of IGF-I was detectable in culture.

Conclusions

Our main conclusions are: a) Free IGF-I content is extremely low in both human serum and synovial fluid and there is no significant difference between the two; b) The concentration of total IGF-I in normal human synovial fluid is an order of magnitude lower than in serum due mainly to the decrease in the concentration of the large complex; c) Preliminary results show that the total IGF-I in osteoarthritic synovial fluids is twice as high as in normal; (d) In normal human cartilage the levels of IGF-I in all its forms are very low and are consistent with the expected exclusion of large molecules by the extracellular matrix; e) By contrast, in osteoarthritic cartilage, the concentrations of all forms of IGF-I are high, probably due to increased permeability of the matrix and binding; and f) The levels of IGF-I found in normal human cartilage are more than an order of magnitude lower than those which stimulate proteoglycan synthesis in human cartilage in culture, whilst the IGF-I levels in osteoarthritic cartilage lie in the range in which stimulation does occur.

References

McQuillan D J, Handley C J, Campbell M A, Bolis S, Milway V E, Herington A C. Stimulation of proteoglycan biosynthesis by serum and insulin-like growth factor-1 in cultured bovine articular cartilage. Biochem J 1986; 240: 423–30.

Schalkwijk J, Joosten L A B, Van den Berg W B, Van Wyk J J, Van de Putte L B A. Insulin-like growth factor stimulation of chondrocyte proteoglycan synthesis by human synovial fluid. Arthritis Rheum 1989; 32(1): 66–71.