INTRODUCTION. As progressive cartilage degeneration seen in osteoarthritis (OA) increases markedly with age, it is important to learn more about potential anabolic factors aimed to prevent or protect cartilage from degradation and/or promote its repair. It is especially important since there is a decline in the ability of chondrocytes to repair with age. As we recently documented (1), human newborn and adult articular chondrocytes endogenously produce one of these factors, osteogenic protein-1 (OP-1), a "repair" factor that has a significant anabolic effect on human and bovine adult articular cartilage. OP-1 induces synthesis of major matrix components, counteracts the catabolic effect of interleukin-1 and fibronectin fragments, promotes cartilage matrix assembly by an up-regulation of CD44 and hyaluronan synthase-2, but has little effect on chondrocyte proliferation. Importantly, our previous data indicate two forms of OP-1 protein in human articular cartilage: an inactive (unprocessed, pro-) form and an active (processed, mature) form. However, only mature, active form has been shown to induce the anabolic effect. The hypothesis has been developed that with aging human articular cartilage shows evidence of a decrease in endogenous OP-1 content, synthesis and metabolism thus leading to an elevated susceptibility of cells to catabolic processes and contributing/promoting cartilage degeneration. In order to test this hypothesis the objective of the current study was to use quantitative approaches to estimate the levels of message and protein of endogenous OP-1 in human adult articular cartilage from donors of different age.

MATERIALS AND METHODS. Full thickness articular cartilage was dissected from 20 human adult donors with no documented history of joint disease through the collaboration with the Regional Organ Bank of Illinois. Tissue was obtained from the load-bearing region of the femoral condyle. Three methods were applied for the quantification of the levels of message and protein of endogenous OP-1 in these samples. OP-1 mRNA expression was measured by using nested RT-PCR method based on the direct extraction of total RNA from tissue. Densities of the PCR bands were evaluated using a Fluor-S Multimager with attached software program and were normalized to the densities of the GAPDH. OP-1 primers utilized in this study were described previously (1). Content of total OP-1 protein was estimated by a newly developed sandwich ELISA and by western blotting. For sandwich ELISA tissue was lyophilized and OP-1 protein was extracted with Lysis buffer, pH 7.5. Human recombinant mature OP-1 and two antibodies, one monoclonal antibody against the entire mature domain of OP-1 and one polyclonal antibody against the synthetic peptide of 18 amino acid close to the N-terminus end, were used for this assay. ELISA results were normalized to the dry weight of the tissue. The same antibodies were applied for western blotting. The densities of specific immunoreactive bands were analyzed with Fluor-S Multimager. Western blot results were normalized to the total protein content.

RESULTS. For these studies on aging only cartilage from normal organ donors with no documented history of joint disease was used. Aliquots of tissue were used for mRNA and protein extraction. The results of semi-quantitative RT-PCR (left graph) indicated the highest levels of mRNA expression in newborn and young adult donors, while with aging this expression was downregulated. When OP-1/GAPDH ratio was plotted onto the graph, results indicated a clear decline in the levels of OP-1 mRNA expression with age. The highest levels of OP-1 message were detected in newborn cartilage. In adult tissues the changes in OP-1 expression had a linear regression and by age of 80 OP-1 message was very low or barely detectable, some donors at old age had the levels of expression even below the detection limit. There were at least 4-5-fold differences in message levels between age of 30 and 80.

Similar results were obtained with quantitative ELISA method (right graph). For the first time we were able to quantify the absolute content of total endogenous OP-1 in human adult articular cartilage in ng quantities by using a method developed by us. We have tested the variety of extraction protocols, the effect of pH and the effect of extractants on the ELISA sensitivity and accuracy. The optimal conditions were selected and by this ELISA method we found that the content of total OP-1 in human normal adult articular cartilage at the age of 40 is between 400 to 600 ng/g of dry tissue. ELISA results supported our PCR data and confirmed the linear decrease in the content of endogenous OP-1 protein as well. A 3-4 fold decrease was observed in a comparison between donors that were over 70 years old to donors around 40. When the same cartilage extracts were analyzed by western blotting using separate antibodies to pro- and mature forms of OP-1, similar pattern in changes of endogenous OP-1 protein was found. Moreover and very important, major differences were noticed on the level of the cleaved mature OP-1. With age the amount of active mature OP-1 was significantly decreased.

DISCUSSION. These studies for the first time report the changes in endogenous OP-1 that occur in human adult articular cartilage with aging on both levels, mRNA and protein. Critically, new quantitative approaches were developed in order to monitor these alterations. As we show, the concentration of endogenous OP-1 protein detected by our ELISA method in human adult articular cartilage is within 100-600 ng/g of dry weight. This content is dramatically decreased with aging when compared to young adults. Interestingly, variations in endogenous OP-1 occur parallel in mRNA and protein levels. Our data indicate that with age there is not only a decrease in total OP-1 available for the anabolic response of chondrocytes, but what is more important, there is a decrease in processed, mature OP-1. Our results indicate a strong correlation between the levels of endogenous OP-1 and the age of human donors supporting the hypothesis that with aging articular cartilage displays a decrease in endogenous anabolic factors, that could contribute to higher susceptibility of articular cartilage to degenerative processes such as seen in OA. The reported ELISA method could be developed as a valuable diagnostic or prognostic tool for predicting the possibility of pathophysiologic changes in connective tissues.


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