YKL-39 (chitinase 3-like protein 2), but not YKL-40 (chitinase 3-like protein 1), is up regulated in osteoarthritic chondrocytes

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Objective: To investigate quantitatively the mRNA expression levels of YKL-40, an established marker of rheumatoid and osteoarthritic cartilage degeneration in synovial fluid and serum, and a closely related molecule YKL-39, in articular chondrocytes.

Methods: cDNA array and online quantitative polymerase chain reaction (PCR) were used to measure mRNA expression levels of YKL-39 and YKL-40 in chondrocytes in normal, early degenerative, and late stage osteoarthritic cartilage samples.

Results: Expression analysis showed high levels of both proteins in normal articular chondrocytes, with lower levels of YKL-39 than YKL-40. Whereas YKL-40 was significantly down regulated in late stage osteoarthritic chondrocytes, YKL-39 was significantly up regulated. In vitro both YKLs were down regulated by interleukin 1β.

Conclusions: The up regulation of YKL-39 in osteoarthritic cartilage suggests that YKL-39 may be a more accurate marker of chondrocyte activation than YKL-40, although it has yet to be established as a suitable marker in synovial fluid and serum. The decreased expression of YKL-40 by osteoarthritic chondrocytes is surprising as increased levels have been reported in rheumatoid and osteoarthritic synovial fluid, where it may derive from activated synovial cells or osteoarthritic tissue or by increased matrix destruction in the osteoarthritic joint. YKL-39 and YKL-40 are potentially interesting marker molecules for arthritic joint disease because they are abundantly expressed by both normal and osteoarthritic chondrocytes.

Biocryhemical markers for the osteoarthritic disease process in synovial or other body fluids are greatly needed for patient management. This includes markers for the diagnosis of early osteoarthritis and for monitoring the disease progress, which is central for the development and testing of new therapeutic regimens. This is particularly true as conventional diagnosis by x ray imaging is an insensitive technique and cannot be used to estimate short term developments.

Several authors have reported that chitinase 3-like protein 1 (YKL-40; cartilage glycoprotein 39), originally described as a major gene product of chondrocytes and synovial cells, as well as one component of neutrophil granules, is clinically useful for the diagnosis of osteoarthritic and rheumatoid arthritis, articular joint disease, being significantly raised in serum and synovial fluid. Contradictory reports exist as to whether normal articular chondrocytes in situ express and secrete YKL-40; thus, by in situ hybridisation no detectable signals were found, whereas YKL-40 was detectable in normal articular chondrocytes by immunostaining. Significant increases of mRNA expression levels have been reported for osteoarthritic chondrocytes.

In our recent gene array experiment we suggested that another related molecule, chitinase 3-like protein 2 (YKL-39, chondrocyte protein 39), is up regulated in osteoarthritic chondrocytes. YKL-39 was originally found to be abundantly secreted by chondrocytes in vitro (about 4% of all secreted proteins). Recently, it has been shown that patients with osteoarthritis or rheumatoid joint disease have autoimmunity against YKL-39. No studies have yet shown whether this molecule might also be a marker for arthritic joint disease.

In this study, we used cDNA array technology with repetitive probes and online quantitative polymerase chain reaction (PCR) technology to try to answer the question as to whether YKL-40 and YKL-39 are markers of osteoarthritic chondrocytes—that is, whether they show increased expression compared with normal chondrocytes within the (normal and diseased) tissue.

MATERIALS AND METHODS

Cartilage samples

For the study of mRNA expression levels, cartilage from human femoral condyles was processed as described previously. For the cDNA array (cDNA) and PCR (PCR) experiments normal articular cartilage (n_ex=20; 43–88 years; n=7; 32–83 years) and early degenerated cartilage (n_ex=21; 43–91 years; n=8; 43–91 years) were obtained from necropsies within 48 hours of death. Osteoarthritic cartilage was obtained from total knee replacement (n_ex=20; 61–84 years; n=8; 63–79 years). Cartilage was considered to be normal if the whole joint showed no significant softening or surface fibrillation of the articular cartilage. Early degenerated cartilage was defined as cartilage derived from a joint which showed overall moderate fibrillation and softening, but no advanced erosion of the articular cartilage. Cases of rheumatoid arthritis were excluded from the study. Only primary degenerated and not regenerative cartilage (osteophytic tissue) was used.

Cell isolation—stimulation with interleukin 1β (IL1β)

For the in vitro culture studies, normal human knee articular cartilage was obtained from normal donors at necropsy within 48 hours of death (n=3; 52–79 years). Cells were isolated and cultured in short term, high density, monolayer cultures as described previously and stimulated with 0 (control), 1, and 10 ng/ml recombinant human IL1β (Biomol, Germany) with 10% fetal calf serum (Biochrom, FRG) for three days.

RNA isolation, cDNA synthesis, conventional PCR

Total RNA from cartilage tissue and cultured chondrocytes was isolated, and cDNA synthesis and conventional PCR (35

Abbreviations: IL1β, interleukin 1β; PCR, polymerase chain reaction
cycles) performed as described previously. Three primer pairs were selected using the PRIMER EXPRESS software (Perkin Elmer): YKL40-forward: ATGGGTTGTGAAGGCGGCTCTTTA CAGG; YKL40-reverse: CTACGTTGCAAGGATGTCCATCTTG; YKL39-forward GCCGGAGCAGCTAGCAAGTCTCTC GGGC; YKL39-reverse: GCCGGCCGCGCGCCACAGGAGCA AAGGGCTCTTC; actin1091.rev: AGCCGCCGATCCACACGGAG.

Statistical analysis of quantitative PCR
Statistical evaluation of significant differences in expression levels in vivo was done by the non-parametric Wilcoxon-Mann-Whitney test.

![Figure 1](PicturePath)
stage osteoarthritic samples showed a significant down regulation of YKL-40 (p<0.005) and up regulation of YKL-39 (p<0.02). YKL-39 was also significantly up regulated in early degenerative lesions (p<0.02) (fig 2A).

**Quantification of mRNA expression levels in normal and osteoarthritic chondrocytes in vivo and in vitro by TAQMAN analysis**

For the quantification of the mRNA levels of YKL-40 and YKL-39 we examined seven normal, eight early degenerative, and eight late stage osteoarthritic cartilage samples. The results confirmed the findings of the cDNA arrays showing that both genes were abundantly expressed in normal and osteoarthritic cartilage with YKL-40 being more abundantly expressed than YKL-39. In late stage osteoarthritic cartilage YKL-40 was down regulated (×4.9; p<0.01), whereas YKL-39 was up regulated (×2.4; p<0.05) (fig 2B).

**DISCUSSION**

It has been suggested that YKL-40 may be one of the best candidate biochemical markers for the diagnosis, prognosis, and monitoring of the osteoarthritic and rheumatoid disease process. Our results were unexpected, however, as YKL-40 was expressed at high levels in normal cartilage, but down regulated in osteoarthritic cartilage. This corrects previous reports in two ways: firstly, normal adult articular chondrocytes do abundantly express YKL-40; secondly, YKL-40 is down regulated and not up regulated in osteoarthritic cartilage. The reason for these discrepancies is somewhat unclear because PCR technology is not needed for abundant gene transcripts such as YKL-40. However, previously used techniques such as in situ hybridisation are always prone to false negative and false positive results.

The increased levels of YKL-40 in osteoarthritic synovial fluid may reflect the increased expression of YKL-40 by other connective tissue cells present within the joints, such as activated synovial fibroblasts or synoviocytes. Indeed, an increased expression of YKL-40 has been shown in rheumatoid synovium. Also, osteophytic tissue, commonly present in degenerated joints, may significantly contribute to the increased levels of YKL-40 in synovial fluid. Alternatively, increased levels of YKL-40 in synovial fluid may reflect increased matrix destruction within the osteoarthritic joint, with increased release of YKL-40 from the cartilage matrix into the synovial fluid. This appears to be a realistic suggestion as YKL-40 is presumably abundant in articular cartilage, at least at the molar level, given its high expression in normal articular chondrocytes in situ.
In contrast with YKL-40, YKL-39 was significantly upregulated in osteoarthritic chondrocytes, in line with our previously reported data. YKL-39 may, therefore, be a more accurate marker of chondrocyte activation in the disease process, as it was also upregulated in the early degenerative cartilage specimens. However, it has yet to be established as a suitable marker for chondrocyte activation when measured in synovial fluid and serum.

Whereas the function of YKL-39 is not at all clear, YKL-40 appears to have a slight positive effect on proliferation and proteoglycan synthesis of articular chondrocytes and a slight proliferative effect on synoviocytes. This may suggest that increased synthesis of YKL-40 by the synovial cells leads to further proliferation of this cell population, which is a typical feature of osteoarthritic synovitis. Possibly, YKL-40, diffusing from the synovial fluid into the articular cartilage, contributes to the enhanced proliferative activity of chondrocytes found in osteoarthritic cartilage. Of note, IL1β downregulated both YKLs, which confirms previous reports on YKL-40.

Overall, YKL-39 and YKL-40 are interesting potential marker molecules for the assessment of arthritic joint disease because they are abundantly expressed by chondrocytes, not only in vitro but also in vivo. However, they are not specific for articular chondrocytes or the osteoarthritic disease process, and YKL-40, in particular, has been shown to be expressed in other organs and other conditions as well. Interestingly, a prognostic value has been attributed to YKL-40 in many different diseases, including cancer and atherosclerosis, suggesting it may be a marker of matrix remodelling in a deranged tissue compartment, a situation which occurs in osteoarthritic joints.

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