CDRAP is expressed in adult articular cartilage, but its expression is not significantly regulated in osteoarthritic chondrocytes

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ABSTRACT
Objectives. In this study we assessed the differential in vivo mRNA expression levels of CDRAP, a potential marker of cartilage degeneration.

Methods. Conventional and real time PCR in a large series of normal (n = 18) and late stage osteoarthritic (n = 24) cartilage specimens were performed.

Results. Conventional PCR analysis could demonstrate the presence of CDRAP mRNA in normal and osteoarthritic cartilages. Real time quantitative PCR confirmed the presence of CDRAP mRNA expression in normal articular chondrocytes in vivo (and in vitro). No significant up-regulation of CDRAP was observed in osteoarthritic chondrocytes in vivo.

Conclusion. The presented results confirm expression of CDRAP by normal and osteoarthritic articular chondrocytes, but indicate that increased expression levels by chondrocytes are not the cause of the increased levels of CDRAP in the synovial fluid of patients with osteoarthritis.

Key words: chondrocytes, gene expression, marker, synovial fluid.

Material and methods
Cartilage samples
Normal human knee articular cartilage from femoral condyles (n = 18; 45 to 88 years; mean age 64.3 years) were obtained from donors at autopsy, within 48 hours of death as described previously (10). Samples from late stage osteoarthritic joint disease were obtained from patients undergoing total knee replacement surgery (n = 24; 60 to 85 years; mean age 72.8 years).

Isolation of primary articular chondrocytes - Long-term monolayer cultures
Normal human knee articular cartilage was obtained from 6 normal donors at autopsy, within 48 hours of death or from amputation (39 yrs - 79 yrs) and cells isolated, seeded out at low density (104 cells/cm2), and cultured as described previously (11).

RNA isolation - cDNA synthesis - conventional PCR
Total RNA from cultured cells and cartilage tissue was isolated, cDNA synthesis and conventional PCR using a cDNA equivalent to 50 ng total RNA were performed as described previously (12). Primers for CDRAP were selected using the PRIMER EXPRESS TM software (Perkin Elmer): forward primer 5´-CCAGCGTTAGGCTGAGCTC-3´, reverse primer 5´-CTGGCTGACCGGAAGCTGGAAGCT-3´.

TAQMAN PCR
The primers (MWG Biotech, Germany) and TAQMAN probes (Eurogentech, Belgium) for CDRAP were designed using PRIMER EXPRESS TM software (Perkin Elmer) and performed as described (11). For CDRAP 300 nM forward primer 5´-GCTGTGGCCCAGCGTTAGGCTGAGCTC-3´ and 3´-CTGGCTGACCGGAAGCTGGAAGCTGGAAGCT-3´.
TTCCAGGACTA-3′), 900 nM reverse primer (5′-AATCTCCGACGCCT-3′), 100 nM detection probe (5′-CCGACTGCCGATTCGACATTGT-3′), and 6.5 mM MgCl₂ were added to the mastermix. All experiments were performed in triplicates.

The assays for types I and II collagens, SOX9 and GAPDH were described previously (11).

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

Results

CDRAP expression in normal and osteoarthritic chondrocytes in vivo - Correlation of CDRAP and collagen type II expression

Conventional PCR (Fig. 1a) as well as real-time PCR (Fig. 1b) did not show any clear difference in between normal and osteoarthritic specimens. No significant correlation was found in between the expression of collagen type II and CDRAP comparing expression levels of both genes in the 50 cases investigated (data not shown).

CDRAP in vitro in primary human articular chondrocytes - Down-regulation during dedifferentiation of chondrocytes

Articular chondrocytes immediately after isolation (non-passaged) expressed CDRAP largely at the same level as chondrocytes in situ (0.02 molecules/molecule GAPDH) whereas chondrocytes cultured in serial subcultures for 3 and 6 months showed a > 10-fold down-regulation in parallel with a loss of the chondrocytic phenotype as reflected by a flattened cell shape (not shown), the down-regulation of chondrocyte-typical COL2A1 and SOX9, and the up-regulation of COL1A1 as marker of the dedifferentiated, fibroblastic phenotype (Fig. 1c).

Discussion

The most important result of our study is that primary adult articular chondrocytes express (low levels of) CDRAP in situ and that this is not increased in osteoarthritic chondrocytes. Thus, also in vivo in the adult, CDRAP is part of the chondrocytic phenotype, which is also documented by its loss during chondrocyte dedifferentiation in culture (6). However, no correlation was found in between the synthetic activity and CDRAP expression levels in the chondrocytes in our study. This fits to the fact that the increased overall synthetic activity of chondrocytes observed in more advanced lesions does not result in elevated levels of CDRAP mRNA expression. Our results support the assumption that the chondrocytes are the source of CDRAP found in synovial fluid, but it also suggests that the increased levels observed in osteoarthriti (7, 9) are not reflecting changes in expression levels within the chondrocytes. More likely this is due to an increased release of resident CDRAP protein from articular cartilage during matrix degradation, in particular as no other cell type except chondrocytes is known to produce CDRAP within the joints. Alternatively, neo-chondrocytes in osteophytic tissue might be the source of synovial fluid CDRAP.

Thus, our study confirms the paradigm of CDRAP as a marker of differentiated adult chondrocytes and suggests that increased levels in diseased synovial fluids reflects degradation-dependent release from the cartilage matrix, but not increased expression by the chondrocytes.

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