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

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ABSTRACT

Objective. *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 chondrocytes. 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 causee of the increased levels of CDRAP in the synovial fluid of patients with osteoarthritis.*

Introduction

Cartilage-derived retinoic acid protein (CDRAP) was originally identified as a chondrocyte gene product repressed by retinoic acid (1) and co-distributing with collagen type II expression during fetal development (2, 3) and fracture healing (4). In adult articular chondrocytes, it was reported to be expressed and regulated by various cytokines and growth factors *in vitro* (5, 6). However, unclear remained the expression levels of CDRAP in primary adult articular chondrocytes *in situ.* Only neo-chondrogenic cartilage was reported to be positive (7). Also, not much is known about the function of CDRAP in adult cartilage and knock-out mice only revealed minor changes in the collagen network on the ultrastructural level (8). Thus, overall, insights in function and importance of CDRAP in chondrocyte biology are rather limited.

More interest in CDRAP arose recently as a potential marker for cartilage disease, both degenerative and arthritic joint disease. Thus, it was reported that in particular in osteoarthritis, elevated levels of CDRAP are observed in synovial fluid, though levels again decreased with advancing disease (7, 9). In this study, we investigated whether a) adult articular chondrocytes express CDRAP in situ (and are, thus, a potential source for CDRAP measured in synovial fluid) and b) whether the elevated levels in synovial fluid are due to increased expression levels of CDRAP in osteoarthritic chondrocytes.

Material and methods

Cartilage samples

Normal 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 (10^4 cells/cm^2), 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´-CTGGCTGACCGGAAGC-TGT-3´, reverse primer 5´-CCAGCG-GTAGGCTGAGCTC-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´-GCTGTGGCCC-

TTCAGGACTA-3´), 900 nM reverse primer (5´-AATCTCCCTGAACGCTGCCT-3´), 100 nM detection probe (5´-CCGACTGCCGATTCCTGACCATTC-3´), and 6.5 mM $MgCl_2$ 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).

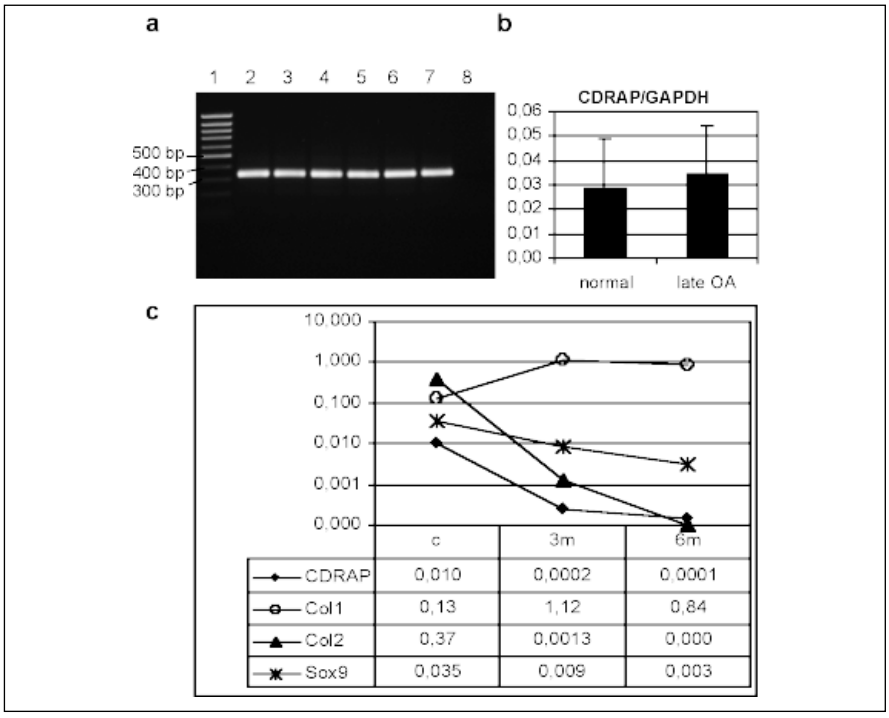

Fig. 1. (a): Demonstration of CDRAP in normal (lanes 2-4) and osteoarthritic (lane 5-7) articular chondrocytes by conventional PCR (lane 1: 100 bp-DNA-ladder, MBI Fermentas, Heidelberg, Germany; lane 8: negative control, no cDNA added; length of amplification product: 326 bp).
(b): Quantificative TAQMAN-analysis for mRNA expression levels of CDRAP in chondrocytes of normal and late-stage osteoarthritic cartilage (given are the values normalized to GAPDH) (standard deviations reflect mRNA measurements in triplicates).
(c): Quantificative TAQMAN-analysis for mRNA expression levels of CDRAP and Col1 and COL2 and SOX9 during in vitro cultures (c: immediately after isolation; three and six months of serial subcultures; cultures were performed from the same donor). Given are the ratios of gene expression levels per GAPDH.

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 osteoarthritis (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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References

1. DIETZ UH, SANDELL LJ: Cloning of a retinoic acid-sensitive mRNA expressed in cartilage and during chondrogenesis. *J Biol Chem* 1996; 271: 3311-6.
2. BOSSERHOFF AK, KONDO S, MOSER M *et al.*: Mouse CD-RAP/MIA gene: structure, chromasomal localization, and expression in cartilage and chondrosarcoma. *Dev Dyn* 1997; 208: 516-25.

3. SANYAL A, CLEMENS V, FITZSIMMONS JS *et al*.: Induction of CD-RAP mRNA during periosteal chondrogenesis. *J Orthop Res* 2003; 21: 296-304.
4. SAKANO S, ZHU Y, SANDELL LJ: Cartilage-derived retinoic acid-sensitive protein and type II collagen expression during fracture healing are potential targets for Sox9 regulation. J *Bone Miner Res* 1999; 14: 1891-901.
5. KONDO S, CHA SH, XIE W-F, SANDELL LJ: Cytokine regulation of cartilage-derived retionic acid-sensitive protein (cd-rap) in primary articular chondrocytes : suppression by Il-1, bfGF, THGβ and stimulation by IGF-1. *J Orthop Res* 2001; 19: 712-9.
6. BOSSERHOFF AK, BUETTNER R: Establishing the protein MIA (melanoma inhibitory activity) as a marker for chondrocyte differentiation. *Biomaterials* 2003; 24: 3229-34.
7. SAITO S, KONDO S, MISHIMA S *et al*.: Analysis of cartilage-derived retinoic-acid-sensitive protein (CD-RAP) in synovial fluid from patients with osteoarthritis and rheumatoid arthritis. *J Bone Joint Surg Br* 2002; 84: 1066-9.
8. MOSER M, BOSSERHOFF AK, HUNZIKER EB, SANDELL LJ, FASSLER R, BUETTNER R: Ultrastructural cartilage abnormalities in MIA/CD-RAP-deficient mice. *Mol Cell Biol* 2002; 22: 1438-45.
9. SCHMIDT-ROHLFING B, SCHNEIDER U, THOMSEN M, BOSSERHOFF AK: Correlation of a novel matrix protein with the degree of cartilage degradation. *Rheumatol Int* 2002; 22: 165-9.
10. BAU B, GEBHARD PM, HAAG J, KNORR T, BARTNIK E, AIGNER T: Relative messenger RNA expression profiling of collagenases and aggrecanases in human articular chondrocytes *in vivo* and *in vitro*. *Arthritis Rheum* 2002; 46: 2648-57.
11. AIGNER T, GEBHARD PM, SCHMID E, BAU B, HARLEY V, PÖSCHL E: SOX9 expression does not correlate with type II collagen expression in adult articular chondrocytes. *Matrix Biol* 2003; 22: 363-72.
12. MCKENNA LA, GEHRSITZ A, SOEDER S, EGER W, KIRCHNER T, AIGNER T: Effective isolation of high quality total RNA from human adult articular cartilage. *Anal Biochem* 2000; 286: 80-5.