CONCISE REPORT

Expression of the matrix receptor CD44v5 on chondrocytes changes with osteoarthritis: an experimental investigation in the rabbit

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Objective: To evaluate the expression of CD44v5 on chondrocytes of hyaline cartilage during the course of osteoarthritis (OA).

Methods: In 12 white New Zealand rabbits the anterior cruciate ligament (ACL) was resected to create an anterior instability of the knee. In 12 control rabbits only a sham operation without resection of the ACL was done. Four animals of each group were killed at 3, 6, and 12 weeks. The loadbearing area was evaluated histologically according to Mankin and by immunostaining for CD44v5.

Results: In the trial group, histological grades of OA showed a positive linear correlation with the time after surgery. Immunostaining showed an increased expression of CD44v5 in the control group after 3 and 6 weeks, which dropped to normal after 12 weeks. There was no difference between control and trial groups after 3 and 6 weeks, but a difference was seen after 12 weeks. A significant positive correlation between CD44v5 expression and the histological grade of OA was found ($r=0.314$).

Conclusions: An in vivo increase of expression of the hyaluronan receptor CD44v5 occurs during the course of OA. Further studies are needed to evaluate whether this pattern applies to man and whether new treatment approaches might evolve from this knowledge.

A transmembrane glycoprotein, CD44, can bind extracellular human cartilage matrix components like fibronectin and collagen types I and IV. Additionally, it has a role as principal cell surface receptor for hyaluronan.[1 2] CD44 occurs in a standard form and in several other isoforms[3] as alternatively spliced variant exon encoded gene products.[4] In human cartilage, CD44H is expressed in chondrocytes of normal and osteoarthritic tissue.[5]

Previous studies have suggested a vital role for chondrocyte CD44 in cartilage homoeostasis and matrix attachment and indicated a participation of CD44v5 in the development of osteoarthritis (OA).[6–8] We have evaluated the expression of the CD44 variant isoform v5 in synovial fluid, synovia, and cartilage of human late stage OA knee joints.[6 7] However, all reports dealt with late stage human OA. Thus, little is known about the expression of CD44v5 during the course of OA.

This study was designed to evaluate (*a*) expression of the isoform CD44v5 during the course of OA and (*b*) possible correlations with histological findings.

MATERIAL AND METHODS

The experiments were performed with permission of the local government in accordance with National Institute of Health (NIH) guidelines (G49/2000). Twenty eight fully grown, female white New Zealand rabbits were used. The animals weighed a mean (SD) of 4193 (299) g at surgery and 4275 (462) g when killed.

All animals were operated on bilaterally under a single general anaesthesia and received an intramuscular depot of antibiotics (Tardomyocel) and intramuscular metamizole (Novalgin) for treatment of pain. A 3 cm medial paramedian skin incision and a medial parapatellar capsular incision were used. In 12 rabbits the anterior cruciate ligament (ACL) was resected (group 1). An intraoperative Lachman test was carried out to evaluate anterior instability. In 12 control rabbits only a sham operation was done (group 2). Postoperatively, all animals were allowed to move freely in their cages (100 cm×70 cm×40 cm). Four animals of each group were killed at 3, 6, and 12 weeks postoperatively. The four animals of group 3 did not undergo any treatment and were killed at the time of surgery of groups 1 and 2. Both distal femurs were immediately stored at −80°C.

Frozen tissue samples (3–5 mm thick) were thawed and fixed with 4% paraformaldehyde for 2 days. After decalcification with buffered EDTA (20% ethylene diamine tetraacetic acid, pH 7.4) the samples were dehydrated and embedded in paraffin. Sections (5 µm thick) were cut, mounted on poly-L-lysine coated glass slides, deparaffinised in xylene, and washed three times with distilled water and then with Tris buffer (pH 7.5) for 3×2 minutes each. Histological changes were evaluated according to the Mankin score[9] by two independent investigators who were unaware of the source of the specimens.

The sections were incubated with proteinase K (ready to use, DAKO, Germany) for 5 minutes, followed by a washing procedure. Endogenous peroxidase activity was quenched by treating tissue sections with 3% H_2O_2 for 10 minutes. After washing, the sections were incubated with 3% bovine serum albumin in Tris buffer to block non-specific binding. After rinsing three times with Tris buffer the sections were next incubated with monoclonal mouse antirabbit CD44v5 (IgG1, clone VFF-18) antibodies at room temperature over night (diluted 1:400, BMS115, Bender MedSystems). The sections were washed three times with Tris buffer and then treated with antimouse IgG (DAKO Envision Systems, Germany, K4001) for 30 minutes at room temperature. To visualise antibody binding, after three washes in Tris, the staining was developed by incubation with AEC-chromogene (ready to use, DAKO, Germany) for 30 minutes, and the reaction was stopped by rinsing in distilled water. In control experiments, (*a*) sections were incubated without primary antibody or (*b*) instead of primary antibody, mouse IgG1 with irrelevant specificity (*Aspergillus niger* glucose oxidase, DAKO, Germany) was used at the same concentration.

Abbreviations: ACL, anterior cruciate ligament; OA, osteoarthritis

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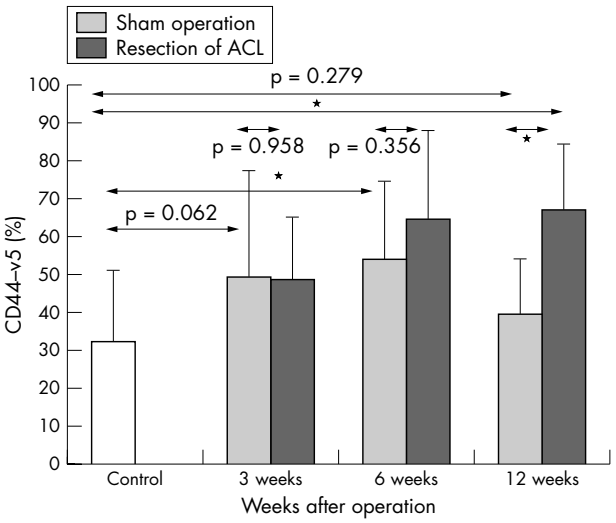

Figure 1 Development of CD44v5 expression during the time course. The percentages of CD44v5 positive cells differed significantly between groups 1 and 2 after 12 weeks ($p<0.05$), but not after 3 ($p=0.958$) and 6 weeks ($p=0.356$). In group 1 (resection of ACL) the percentage of CD44v5 positive cells increased steadily in the time course, whereas in group 2 (sham operation) it was significantly raised after 6 weeks but decreased from 6 to 12 weeks. After 12 weeks there was no difference in the percentage of CD44v5 positive cells between group 2 (sham operation) and the unoperated controls ($p=0.279$), whereas the percentage of CD44v5 positive cells was increased in group 1 (resection of ACL) (*$p>0.05$).

Histological sections were photographed (CoolSnap-Pro Color A00J82025, Media Cybernetics, Silver Spring, MD, USA). The cells were counted using the Image-Pro Plus software for Windows, version 4.1 (Media Cybernetics, Silver Spring, MD, USA). The total number of chondrocytes was determined in a haematoxylin and eosin stained section and compared with the number of immunohistochemically stained cells.

Statistical analysis was performed using SPSS 11.0 (SPSS GmbH, Munich, Germany). Student's *t* test was used for comparison of scores, and Pearson's coefficient was calculated for correlations.

RESULTS

Two animals developed postoperative haematomas—one a superficial wound infection treated with a single shot antibiotic, and one animal a wound gap reoperated on 2 days after surgery. All four animals were included in the analysis. At the time of death, one animal of group 1 had intact ACLs and was excluded from evaluation. In total, 54 joints were evaluated.

Histological grading of OA

According to the Mankin score, group 1 showed a highly significant increase in the grade of OA in comparison with groups 2 and 3. In group 3 the mean (SD) grade of OA was 1.75 (1.58). In group 2 it was 3.25 (0.71) after 3 weeks, 1 (1.20) after 6 weeks, and 2.25 (1.17) after 12 weeks. In group 1 the scores were 7.25 (3.62) after 3 weeks, 8 (2.88) after 6 weeks, and 9.83 (3.06) after 12 weeks. Group 1 had significantly higher values than group 2 at every time point ($p<0.01$). The average histological grade of OA increased with the number of postoperative weeks.

Immunohistochemical staining of CD44v5

CD44v5 positive chondrocytes were found in the trial and control groups (see figs 2A–F). In group 2 the mean (SD) percentage of CD44v5 positive cells was 49.1 (28.1)% after 3 weeks, 54.1 (20.4)% after 6 weeks, and 39.5 (14/7)% after 12 weeks (fig 1). In group 1 the percentages were 48.5 (16.6)% after 3 weeks, 64.6 (23.5)% after 6 weeks, and 66.9 (17.6)% after 12 weeks. The percentages of CD44v5 positive cells differed significantly between the two groups after 12 ($p<0.05$), but not after 3 ($p=0.958$) and 6 weeks ($p=0.356$). In group 3 the mean (SD) percentage of CD44v5 positive cells was 32.2 (19.0)%. In group 1 the percentage of CD44v5 positive cells increased steadily with time, whereas in group 2 it was significantly raised compared with group 3 ($p<0.05$) after 6 weeks but decreased from 6 to 12 weeks. After 12 weeks there was no difference between groups 2 and 3 ($p=0.279$), whereas the percentage of CD44v5 positive cells was increased in group 1 ($p>0.05$).

A significant correlation was found between the percentage of CD44v5 positive chondrocytes and the histological grade ($r=0.314$, $p=0.021$).

DISCUSSION

As far as we know, this study is the first to evaluate the expression of CD44 containing variant isoform v5 and its correlation with histological features in experimental OA.

A percentage of chondrocytes in healthy cartilage expressed CD44v5, indicating its physiological role. CD44v5 positive and CD44v5 negative chondrocytes were found in most of the specimens. This corresponds with findings of the standard isoform CD44H on human OA cartilage[5 10] and is in accordance with the detection of alternative spliced messenger RNA transcripts containing the exon 5 in OA chondrocytes.[5 10] In human OA, up regulated CD44H expression was found in the deep zone of the cartilage.[10] This finding correlates with our late stage results after 12 weeks.

The increase of CD44v5 expression with increasing OA stages suggests that it participates in the pathogenesis of OA. This is in line with the results of others, who showed that up regulated chondrocyte metabolism leads to up regulated CD44 expression in cultured bovine chondrocytes.[11] Another explanation for an increased CD44v5 expression might be found in the obviously altered binding capacity compared with CD44H. In tumour cells a correlation of increased ectopic CD44v5–7 expression on the cell surface and an increased soluble hyaluronan binding capacity was seen.[12] The authors postulated that the regulation of clustering of CD44, mediated by factors including the presence of variant exons and glycosylation, allows cells to regulate their hyaluronan binding capacities.

In a previous study on the expression of CD44v5 in synovial fluid, synovia, and cartilage of human late stage OA, we demonstrated that CD44v5 expression in cartilage and synovia seemed to correlate strongly with the histological grade of cartilage destruction.[6] In the present study, we confirmed a correlation of chondrocyte CD44v5 expression and histological joint destruction, although correlation coefficients were low. Reasons for this partial correspondence might be found in different evaluation methods (percentage of patients expressing CD44v5 *v* percentage of chondrocytes expressing CD44v5) and inclusion of healthy cartilage and early stage OA in the present results, in contrast with predominantly late stage OA specimens.[6]

In patients with rheumatoid arthritis, serum and synovial fluid sCD44v5 levels correlated with advanced stages and inflammatory activity.[13–15] We found evidence that CD44v5 expression is, at least partly, influenced by inflammation, as the sham operation group showed increased expression after 6 weeks, which returned to normal after 12 weeks.

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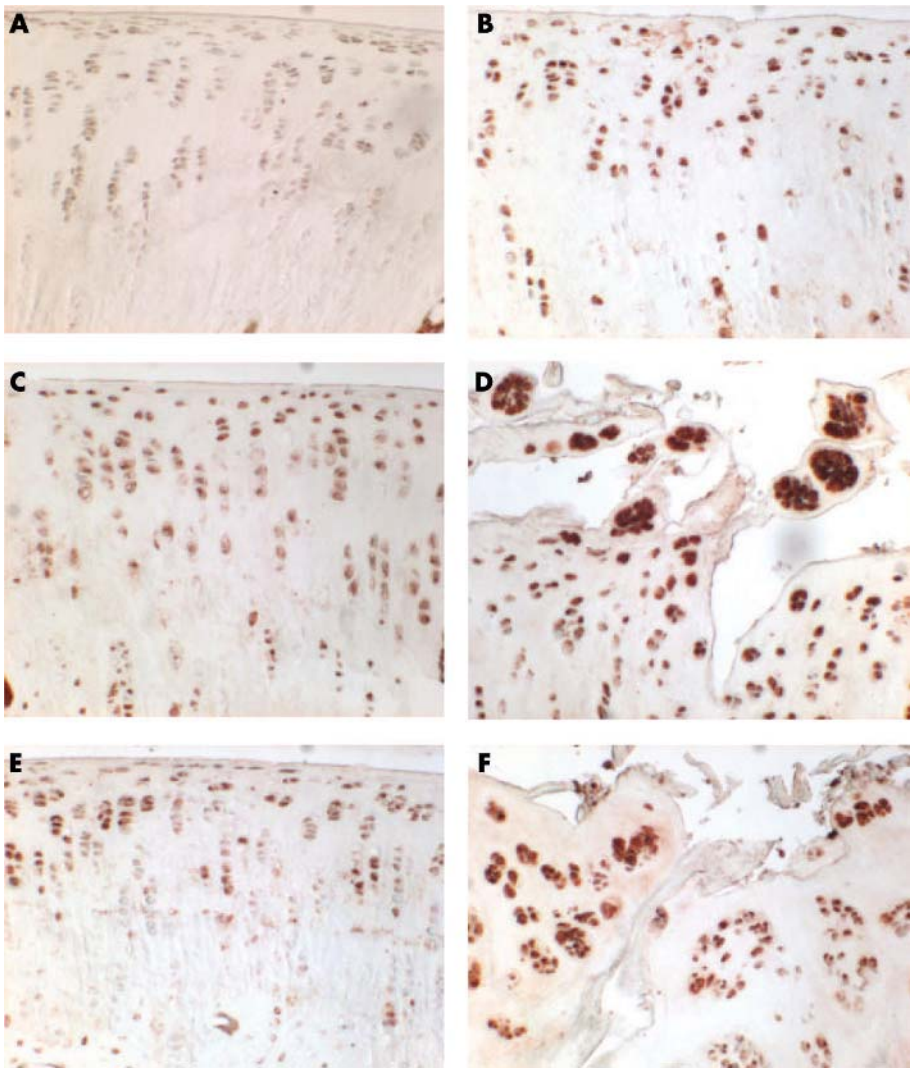

Figure 2 CD44v5 expression (A) 3 weeks after sham operation; (B) 3 weeks after ACL resection; (C) 6 weeks after sham operation; (D) 6 weeks after ACL resection; (E) 12 weeks after sham operation; (F) 12 weeks after ACL resection;

In conclusion, our results show an increased expression of CD44v5 during the course of OA. Further studies are needed to evaluate (*a*) its physiological role, (*b*) whether CD44v5 up regulation is an underlying cause of OA or a repair response, (*c*) whether this applies to humans, and (*d*) whether new treatment approaches might evolve from this knowledge.

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REFERENCES

1 **Aruffo A**, Stamenkovic I, Melnick M, Underhill CB, Seed B. CD44 is the principal cell surface receptor for hyaluronate. *Cell* 1990;**61**:1303–13.
2 **Culty M**, Miyake K, Kincade PW, Sikorski E, Butcher EC, Underhill C, *et al*. The hyaluronate receptor is a member of the CD44 (H-CAM) family of cell surface glycoproteins. *J Cell Biol* 1990;**111**:2765–74.
3 **Bajorath J**. Molecular organization, structural features, and ligand binding characteristics of CD44, a highly variable cell surface glycoprotein with multiple functions. *Proteins* 2000;**39**:103–11.
4 **Hale LP**, Haynes BF, McCachren SS. Expression of CD44 variants in human inflammatory synovitis. *J Clin Immunol* 1995;**15**:300–11.
5 **Salter DM**, Godolphin JL, Gourlay MS, Lawson MF, Hughes DE, Dunne E. Analysis of human articular chondrocyte CD44 isoform expression and function in health and disease. *J Pathol* 1996;**179**:396–402.
6 **Fuchs S**, Dankbar B, Wildenau G, Goetz W, Lohmann CH, Tibesku CO. Expression of the CD44 variant isoform 5 in the human osteoarthritic knee joint: correlation with radiological, histomorphological, and biochemical parameters. *J Orthop Res* 2004;**22**:774–80.
7 **Fuchs S**, Rolauffs B, Arndt S, Tibesku CO, Prehm P. CD44H and the isoforms CD44v5 and CD44v6 in the synovial fluid of the osteoarthritic human knee joint. *Osteoarthritis Cartilage* 2003;**11**:839–44.
8 **Knudson W**, Loeser RF. CD44 and integrin matrix receptors participate in cartilage homeostasis. *Cell Mol Life Sci* 2002;**59**:36–44.
9 **Mankin HJ**, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. II. Correlation of morphology with biochemical and metabolic data. *J Bone Joint Surg Am* 1971;**53**:523–37.

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10 **Ostergaard K**, Salter DM, Andersen CB, Petersen J, Bendtzen K. CD44 expression is up-regulated in the deep zone of osteoarthritic cartilage from human femoral heads. *Histopathology* 1997;**31**:451–9.
11 **Chow G**, Knudson CB, Homandberg G, Knudson W. Increased expression of CD44 in bovine articular chondrocytes by catabolic cellular mediators. *J Biol Chem* 1995;**270**:27734–41.
12 **Sleeman J**, Rudy W, Hofmann M, Moll J, Herrlich P, Ponta H. Regulated clustering of variant CD44 proteins increases their hyaluronate binding capacity. *J Cell Biol* 1996;**135**:1139–50.
13 **Feyertag J**, Haberhauer G, Skoumal M, Kittl EM, Bauer K, Dunky A. [Serum soluble CD44 isoform variant 5 level in patients with seropositive rheumatoid arthritis treated with cyclosporin A]. *Acta Med Austriaca* 2000;**27**:156–9.
14 **Haberhauer G**, Kittl EM. Soluble CD44 isoform variant-5 (sCD44v5): a new serum marker in rheumatoid arthritis? *J Rheumatol* 1998;**25**:1442–4.
15 **Kittl EM**, Haberhauer G, Ruckser R, Selleny S, Rech-Weichselbraun I, Hinterberger W, *et al*. Serum levels of soluble CD44 variant isoforms are elevated in rheumatoid arthritis. *Rheumatol Int* 1997;**16**:181–6.

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