

EXTENDED REPORT

Galectin-3 surface expression on human adult chondrocytes: a potential substrate for collagenase-3

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Background: Galectin-3 is a lectin detected in mature and early hypertrophic chondrocytes; osteoarthritic (OA) chondrocytes can re-express hypertrophic markers.**Objective:** To investigate the synthesis and subcellular localisation of galectin-3 in adult chondrocytes as well as the possibility of cleavage of galectin-3 by collagenase-1 and -3.**Methods:** Galectin-3 was assessed by immunohistochemistry and real time polymerase chain reaction (PCR) in normal and OA cartilage. Its localisation was investigated by subcellular fractionation, immunocytochemistry, and flow cytometry. Proteolysis of galectin-3 by collagenase-1 and -3 was determined by *in vitro* assay.**Results:** Galectin-3 expression was increased 2.4-fold as measured by reverse transcriptase (RT)-PCR ($p < 0.05$, $n = 5$) and threefold by immunohistochemistry ($p < 0.003$, $n = 6$) in OA cartilage compared with normal cartilage. In adult chondrocytes, galectin-3 was found in the cytosol and membrane enriched fractions. Both immunocytochemistry and flow cytometry confirmed the presence of galectin-3 at the surface of chondrocytes. A strong correlation was found between integrin- $\beta 1$ and galectin-3 expression at the surface of chondrocytes. Moreover, collagenase-3 cleaved galectin-3 with a higher activity than collagenase-1. The proteolysed sites generated were identical to those produced by gelatinases A and B.**Conclusion:** Galectin-3 may play a part in OA, having two roles, one intracellular and not yet identified, and another at the cell surface, possibly related to the interaction of chondrocytes and the cartilage matrix.

Abnormality in cartilage is age related and, consequently, osteoarthritis (OA) is one of the most prevalent diseases in older people.^{1–2} OA involves both a decreased reparative process and an increased degradative phenomenon, thereby limiting the capacity for articular cartilage repair.^{3–4} The metabolic modifications of chondrocytes that occur during the onset of OA are related both to new stimuli (proinflammatory cytokines, growth factors from subchondral bone) and to changes in the cartilage extracellular matrix (ECM) composition. These metabolic modifications lead chondrocytes to re-express proteins normally produced in hypertrophic chondrocytes during bone development. Galectin-3 is one of these molecules and its expression seems to be highly regulated in this process.

Galectin-3 is an animal lectin that belongs to the galectin superfamily. Galectins, like other lectins, recognise a glycosylation structure with neither enzymatic nor immune activity.⁵ The galectin-3 gene, first identified as the macrophage marker 2 protein, was mapped on human chromosome 14 (14q21–22). This gene has 17 kb and is composed of six exons and five introns and codes for a soluble protein of 30 kDa.^{6–7} Although first discovered in macrophages, galectin-3 has been found to be more widely distributed in tissues, which include the gut, brain, kidneys, and skeleton.⁸ Reported data also suggest that galectin-3, depending on its subcellular localisation, participates in a variety of processes such as RNA splicing,⁹ differentiation,¹⁰ apoptosis,^{11–13} and cell-cell or cell-matrix interactions.^{14–17} Among different extracellular ligands of galectin-3,^{17–20} fibronectin and integrins, which are increased in OA cartilage, may have a role in modifying diseased chondrocyte metabolism.^{21–22} In addition, galectin-3 has been identified as an advanced glycation end product receptor,²³ and glycation end products are involved in OA.^{24–25} Moreover, the proteolysis of galectin-3

by metalloproteinases (MMPs), such as gelatinase A (MMP-2) or gelatinase B (MMP-9), can modify these interactions when galectin-3 is expressed at the cell surface.^{17–26}

In bone development, galectin-3 is found in both osteoblasts^{27–28} and osteoclasts,²⁸ as well as in chondrocytes.^{27–28} More particularly, galectin-3 is expressed in the early hypertrophic chondrocytes of the growth plate; however, it is rarely found in the late hypertrophic chondrocytes undergoing terminal maturation in the calcified zone.^{29–30} A knockout mouse model has indicated that galectin-3 is implicated in the chondrocyte death and vascular invasion coupling process. These results suggest a role for galectin-3 as a regulator of chondrocyte survival.³¹

Because OA chondrocytes re-express hypertrophic genes, we first investigated the presence of galectin-3 in human normal and OA cartilage. Then, we focused on the galectin-3 subcellular localisation in chondrocytes and more particularly at the cell surface. Moreover, we investigated the capacity of collagenase-1 and -3 to cleave galectin-3.

MATERIALS AND METHODS

Specimen selection

Human chondrocytes from articular cartilage (femoral condyles and tibial plateaus) were obtained from 13 normal donors (mean (SD) age 63 (13)) and 15 patients with OA (aged 65 (7)) who had undergone total knee arthroplasty. All patients with OA were evaluated by a certified rheumatologist and were diagnosed as having OA based on the criteria

Abbreviations: 7-AAD, 7-aminoactinomycin D; ECM, extracellular matrix; MMP, matrix metalloproteinase; OA, osteoarthritis/osteoarthritic; PBS, phosphate buffered saline; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase-polymerase chain reaction; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis

developed by the American College of Rheumatology Diagnostic Subcommittee for OA.³²

Chondrocyte culture

Chondrocytes were released from the articular cartilage by sequential enzymatic digestion at 37°C as previously described³³ and cultured in Dulbecco's modified Eagle's medium (Gibco-BRL, Burlington, Ontario, Canada) supplemented with 10% heat inactivated fetal bovine serum (Gibco-BRL) and an antibiotic mixture (100 U/ml penicillin base, 100 µg/ml streptomycin base; Gibco-BRL) in a humidified atmosphere of 5% CO₂/95% air. First passage OA chondrocytes were used in all experiments.

Immunohistochemistry detection of galectin-3

Cartilage specimens from normal subjects and patients with OA were processed for immunohistochemical analysis as previously described.^{34, 35} Briefly, specimens were fixed in TissuFix No 2 (Laboratoires Gilles Chaput, Montreal, Quebec, Canada) for 24 hours, then embedded in paraffin. Sections (5 µm) of paraffin embedded specimens were placed on Superfrost Plus slides (Fisher Scientific, Nepean, Ontario, Canada), deparaffinised in toluene, rehydrated in a graded series of ethanol, and preincubated with chondroitinase ABC (0.25 U/ml; Sigma-Aldrich, Canada) in phosphate buffered saline (PBS) pH 8.0 for 60 minutes at 37°C. After this, the specimens were washed in PBS, then placed in 0.3% hydrogen peroxide/PBS for 15 minutes. Slides were further incubated with a blocking serum for 60 minutes, blotted, and then overlaid with a rabbit serum containing primary polyclonal antibodies against galectin-3 (Covalab, Oullins, France; dilution 1/2500) for 18 hours at 4°C in a humidified chamber. Each slide was washed three times in PBS (pH 7.4) and stained using the avidin-biotin complex method (Vectastain ABC kit; DAKO Diagnostics, Canada). This method entails incubation in the presence of the biotin conjugated secondary antibody for 45 minutes at room temperature followed by the addition of the avidin-biotin-peroxidase complex for 45 minutes. All incubations were carried out in a humidified chamber at room temperature and the colour developed with a 3,3'-diaminobenzidine (DAKO Diagnostics, Canada) containing hydroxide peroxide. Slides were counterstained with saffron. To determine the specificity of staining, slides were incubated with a rabbit non-immune serum (Vector Laboratories Inc, Burlingame, CA). Several sections were made from each block of cartilage and slides from each specimen were processed for immunohistochemical analysis. Each section was examined under a light microscope (Leica DMLS, Leica, Weitzlar, Germany) and photographed with CoolSnap CF and RS image software (Roper Scientific Inc. Tucson, AZ).

Morphometric analysis

The antigen in cartilage was quantified as described previously.^{34, 36} The presence of the antigen was estimated by determining the number of chondrocytes staining positive in the entire thickness of cartilage. Cartilage was divided into three microscopic fields (×20; Leitz Diaplan) and the results averaged. For each arthritic specimen, it was ensured before evaluation that an intact cartilage surface could be detected and used as a marker for validation of morphometric analysis. The total number of chondrocytes and the number of chondrocytes staining positive for the specific antigen were determined. The final results were expressed as the percentage of chondrocytes staining positive for the antigen (cell score), with the maximum score being 100%. Values are expressed as median and range. Statistical analysis was performed using the Mann-Whitney U test. Values of $p < 0.05$ were considered significant.

Quantitative polymerase chain reaction (PCR)

RNA extraction

Human articular cartilage was homogenised in Trizol reagent, followed by the addition of chloroform as described.³⁷ After being shaken and cooled at 4°C for 1 hour, the solution was centrifuged (12 000 *g*, 2 hours at 4°C). The aqueous phase was transferred to a new tube and precipitated with one volume of isopropyl alcohol overnight at -20°C. After centrifugation (12 000 *g*, 30 minutes at 4°C), the pellet was resuspended in QIAGEN lysis buffer from the RNeasy Plant mini-kit and extracted according to the manufacturer's protocol (QIAGEN Inc, Mississauga, ON, Canada). RNA was measured by the RiboGreen[®] RNA reagent (Molecular Probe, Eugene, OR).

Reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA (2 µg) was used in the RT-PCR reaction. The assay was carried out in a Gene ATAQ controller (Amersham Pharmacia Biotech, Baie d'Urfé, PQ, Canada). The RNA was converted to single stranded DNA with 50 units of M-MLV reverse transcriptase (InVitrogen) in 20 µl of reaction mixture containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 5 mM MgCl₂, 1 mM of each dNTP (Amersham Pharmacia Biotech), 2.5 µM random hexamers (Amersham Pharmacia Biotech), and 20 units RNase inhibitor. The mixture was incubated at 42°C for 45 minutes and at 99°C for 5 minutes. Real time PCR on a Gene Amp 5700 Sequence Detector System (Applied BioSystems, Foster City, CA) was performed in a total volume of 50 µl in the presence of 10 ng 18S cDNA or 100 ng galectin-3 cDNA. Water was used as a negative control. MgCl₂ was added to a final concentration of 2 mmol/l, each oligonucleotide primer added to a final concentration of 100 nmol/l, and the SYBR[®] Green core reagents added according to the manufacturer's instructions (Applied BioSystems). To avoid sample contamination from previous amplifications, an initial step with AmpErase UNG was performed for 2 minutes at 50°C followed by an AmpErase inactivation (10 minutes at 95°C). PCR was performed with an initial step for 5 minutes at 95°C followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. We verified that a single peak was obtained for each product using the Gene Amp 5700 Sequence Detector System software. PCR primers for galectin-3 were sense 5'-GGCCACTGATTGTGCCTTAT and antisense 5'-TCTTCTCCCTTCCCAGT. These primers were designed using Primer3 software (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi; accessed 10 March 2004) and produced a 224 bp amplified fragment. Specific primers for 18S were sense 5'-GAATCAGGGTTCGATCCG and antisense 5'-CCAAGATCCAACACTACGAGC, which gave a 279 bp amplified fragment. Primers were synthesised by Alpha DNA (Montreal, Quebec, PQ, Canada). Data analysis was carried out using the comparative Ct method with the Gene Amp 5700 Sequence Detector System software. The level of galectin-3 expression was normalised to 18S. Statistical analysis was performed using Student's *t* test. Values of $p < 0.05$ were considered significant.

Cell fractionation

OA chondrocytes were recovered with a cell scraper in a hypotonic buffer (20 mM Tris HCl, 50 mM NaCl, pH 7.5). Cells were submitted to a freeze-thaw cycle, sonicated at 40 V (Vibra cell, Sonics & Material Inc, Danbury, CT) for 20 seconds, and then centrifuged at 200 *g*. Pellets containing nuclei were saved while supernatants were centrifuged at 100 000 *g*. Pellets obtained after this step contained membrane enriched fractions, whereas supernatants were composed of cytosol. Pellets were then resuspended in 20 mM Tris HCl, 50 mM NaCl, pH 7.5, containing 0.1% of Nonidet

P40. Proteins of each fraction were assessed with the bicinchoninic acid method³⁸ and analysed using western blotting with the anti-galectin-3 antibody (dilution 1/40 000) and a monoclonal anti-integrin- β 1 (Chemicon International Inc, Temecula, CA; dilution 1/5000)

Immunocytochemistry

OA chondrocytes were seeded in eight well Lab-Tek chamber slides (Nunc, Naperville, IL) at a density of 30 000 cells/cm². Cells were incubated overnight, washed twice in PBS, and fixed in TissueFix No 2. Immunostaining was performed and analysed with both optical and fluorescent microscopy. After fixation, slides were rinsed three times in PBS. For cell permeabilisation, some slides were incubated in PBS containing 0.3% Triton X100 for 30 minutes and then rinsed three times in PBS. After blocking in a humidified chamber with 1.5% goat serum (Vector Laboratories Inc) in PBS for 1 hour, the polyclonal anti-galectin-3 antibody was incubated over-

night at a dilution of 1/10 000. Controls were performed with non-immune rabbit serum (Vector Laboratories Inc). For fluorescent microscopy, an Alexa conjugated antirabbit IgG (Molecular probes, Eugene, OR) was used at a dilution of 1/5000. Cells were examined under a Nikon Eclipse TE300 fluorescent microscope. Images were recorded with a CCD camera (Roper Scientific Inc) and Metafluor software (University Imaging Corp). Three independent experiments were performed.

Flow cytometry

Primary human OA chondrocytes were briefly trypsinised, rinsed in PBS containing 1.5% goat serum, and incubated with either the anti-galectin-3 antibody (dilution 1/100) or with the monoclonal anti-integrin- β 1 (dilution 1/250) for 1 hour. Non-immune serum was used at the same concentration and served as control. After rinsing, cells were incubated in a secondary antibody conjugated to

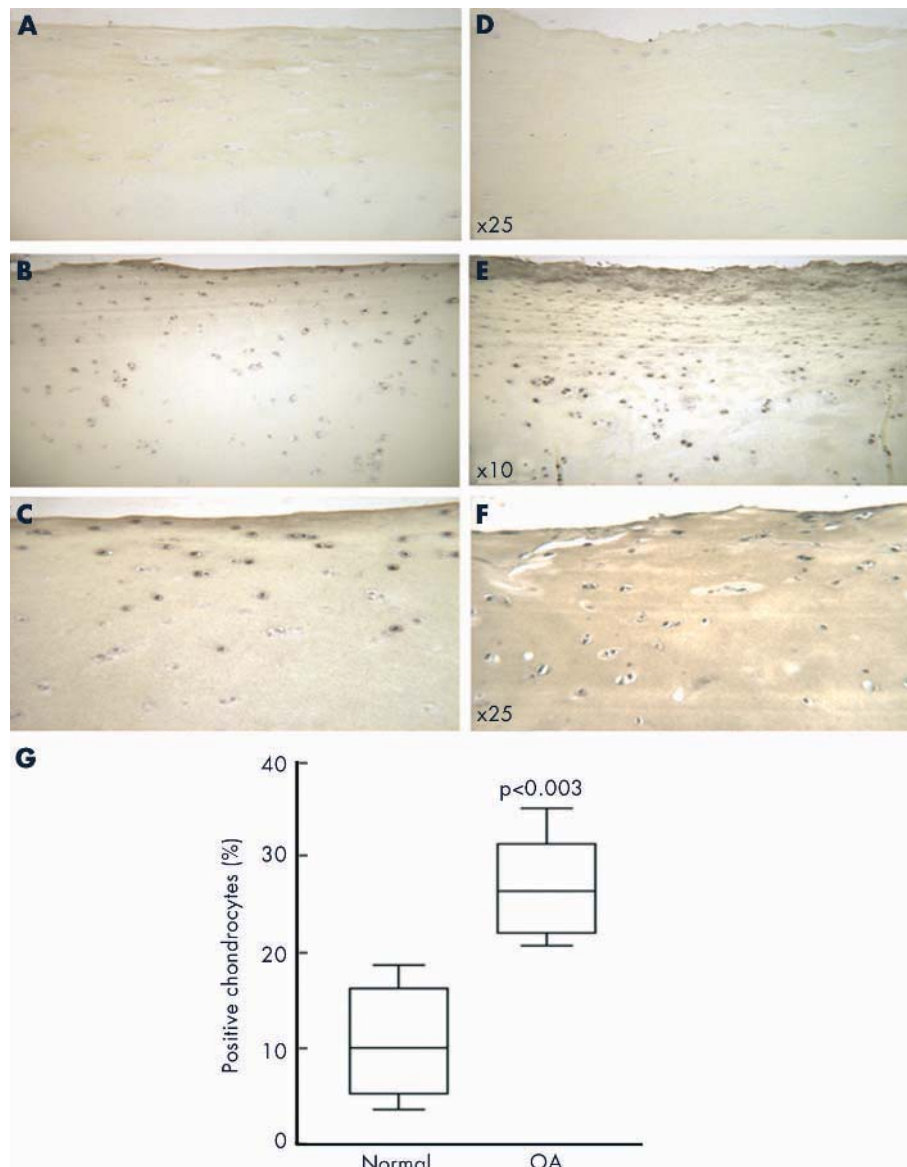


Figure 1 Representative section of cartilage, showing immunostaining for galectin-3 from normal (A–C) and OA cartilage (D–F). (A and D) Non-immune serum; (B, C, E, and F) polyclonal anti-galectin-3. Original magnification $\times 25$; (G) galectin-3 levels over the entire cartilage thickness. Data are presented as box plots, where the boxes represent the first and third quartiles, the lines within the boxes represent the median, and the lines outside the boxes represent the spread of the galectin-3 cell scores outside the first and third quartiles. The p value, obtained with the Mann-Whitney test, indicates the difference compared with the normal group.

phycoerythrin (dilution 1/100; Jakson Immunoresearch Laboratories, West Grove, PA) for 30 minutes and then rinsed in PBS for 10 minutes before samples were read in a FACSscan (Becton-Dickinson, Oakville, ON, Canada). A cell non-permeable stain, 7-aminoactinomycin D (7-AAD; Molecular Probes), was added 5 minutes before reading to discriminate permeabilised cells from whole cells.

Proteolysis analysis of galectin-3

Human recombinant galectin-3, generated as previously described,³⁹ was incubated with either activated human recombinant collagenase-1 or -3 or gelatinase A at a molecular ratio of 1/50 and 1/10 for 30 minutes at 37°C in 50 mM Tris-HCl buffer, pH 7.5, containing 150 mM NaCl and 10 mM CaCl₂. Incubation was stopped with a volume of 6×denaturing Laemmli buffer, and the entire sample was loaded on a 4–12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) system. After migration, proteins were detected with silver nitrate staining (Bio-Rad; Mississauga, Ontario, Canada).

Sequencing of galectin-3 fragments

Recombinant galectin-3 was digested by collagenase-3 as above. Fragments were separated by 4–12% SDS-PAGE and blotted for 1.5 hours at 180 mA onto a PVDF membrane using 10 mM CAPS, pH 10.8, containing 10% methanol as a transfer buffer. Fragments were detected using Coomassie blue staining and bands were cut and analysed for amino acid sequence (Sheldon Biotechnology Centre, McGill University, Montreal, Quebec, Canada).

RESULTS

Galectin-3 expression by human normal and OA chondrocytes

OA chondrocytes are often compared with hypertrophic-like chondrocytes owing to their ability to express several proteins also found in developing bone. For example, OA chondrocytes re-express annexins II and V,^{40–41} collagenase-3,^{42–43} osteopontin,⁴⁴ and type X collagen.⁴⁵ In this study we investigated the capacity of human normal and OA chondrocytes to synthesise galectin-3, which was previously described as expressed by hypertrophic chondrocytes during bone development.^{29–30} Data obtained by immunohistochemistry showed that galectin-3 was expressed by normal (figs 1B and C) and OA cartilage (figs 1E and F). In contrast, non-immune serum showed no signal (figs 1A and D). Moreover,

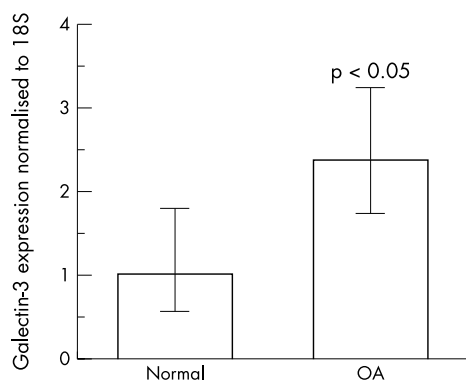


Figure 2 Expression of galectin-3 mRNA in human normal and OA cartilage was studied using real time RT-PCR as described in "Materials and methods". The PCR analysis was performed by normalising the PCR products of the galectin-3 to the 18S PCR products. Bars show the mean (SD) intensity of five normal and OA specimens. The p value, obtained with Student's *t* test, indicates the difference compared with the normal group.

the quantitative measurement of galectin-3 staining (fig 1G) was significantly increased in OA cartilage (median 26.5% (range 20.8–35.2)) compared with normal cartilage (10.1% (3.6–18.8), $p < 0.003$, $n = 6$). As illustrated in fig 2, we confirmed, using real time PCR, that galectin-3 mRNA was expressed at a higher level in OA than normal cartilage (2.4-fold, $p < 0.05$, $n = 5$).

Subcellular distribution of galectin-3 in OA chondrocytes

As the function of galectin-3 depends on its subcellular localisation, we determined its localisation using a subcellular fractionation methodology. Data showed that galectin-3 was present in cytosol as well as in nuclear and membrane enriched fractions (fig 3). This result corroborated the main localisations of galectin-3 in different cell lines.^{46–50} However, up to now galectin-3 has never been found in the plasma membranes of chondrocytes. The quality of the membrane enriched fraction was assessed using a specific marker of plasma membrane. Integrin- β 1 was chosen because it was previously shown to be expressed at the surface of chondrocytes.^{51–52} As represented in fig 3, a stronger signal was obtained with the anti-integrin- β 1, in the fraction enriched with plasma membrane, indicating that this part of the fractionation was of good quality. In contrast, some integrin- β 1 was also detected in the nuclear fraction, suggesting that non-lysed cells might contaminate this fraction. Therefore we analysed the nuclear staining in confocal microscopy by colocalising, in permeabilised cells, galectin-3 and Sytox green, a nuclear marker. No galectin-3 was found in the nuclei (data not shown, $n = 2$).

To verify the new localisation of galectin-3 at the surface of adult chondrocytes, we pursued our studies using immunocytology. Cells were either permeabilised or non-permeabilised by using or not 0.3% of Triton X100 before the incubation with the anti-galectin-3 antibody. As illustrated in Figure 4, no staining was noted with the non-immune serum (figs 4A and C). Furthermore, data demonstrated a weaker signal in non-permeabilised cells (fig 4B) than in permeabilised cells (figs 4D), which again suggests the

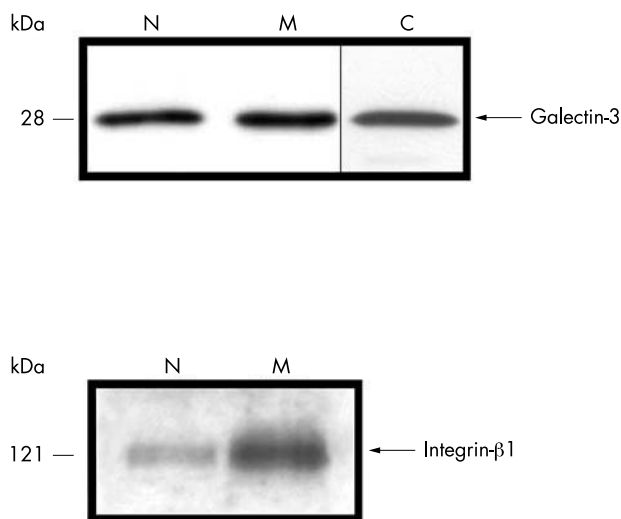


Figure 3 Subcellular OA chondrocyte fractionation was performed as described in "Materials and methods". Proteins were quantified for each fraction, 10 μ g were blotted, and immunodetection was performed with either a polyclonal anti-galectin-3 or a monoclonal anti-integrin- β 1 antibody. N, nuclear fraction; M, membrane enriched fraction; C, cytosol.

localisation of galectin-3 at the chondrocyte surface, confirming the results from fig 3.

To rule out definitively the possibility of membrane permeabilisation during sample preparation (as could, for example, occur in the fixative step), we decided to use flow cytometry. Indeed, this methodology allowed the sorting of non-permeabilised and permeabilised cells by using 7-AAD staining, which can penetrate the cells only when the plasma membrane is disrupted. Results showed that only 5% of total cells had a disrupted membrane at the end of incubations in the flow cytometry experiments (data not shown). Therefore, 95% of chondrocytes in these preparations were used to analyse the surface labelling. As expected, data for intact cells showed a shift of the phycoerythrin signal in the presence of the anti-galectin-3 antibody when compared with the cells

treated with the non-immune serum (fig 4E). This finding definitively demonstrated that galectin-3 was expressed at the surface of adult chondrocytes. Because Ochieng *et al* had previously demonstrated interactions between galectin-3 and integrins,¹⁵ we investigated whether these two proteins could be related in chondrocytes. Therefore, by using flow cytometry, we compared the integrin- β 1 expression with the expression of galectin-3 measured at the chondrocyte surface. A strong correlation ($R^2 = 0.8699$, $n = 6$) was seen regardless of whether samples came from normal subjects or patients with OA (fig 5).

Proteolytic activity of collagenase-3 on galectin-3

It has been previously shown that when galectin-3 is expressed at the cell surface its N-terminal domain is a

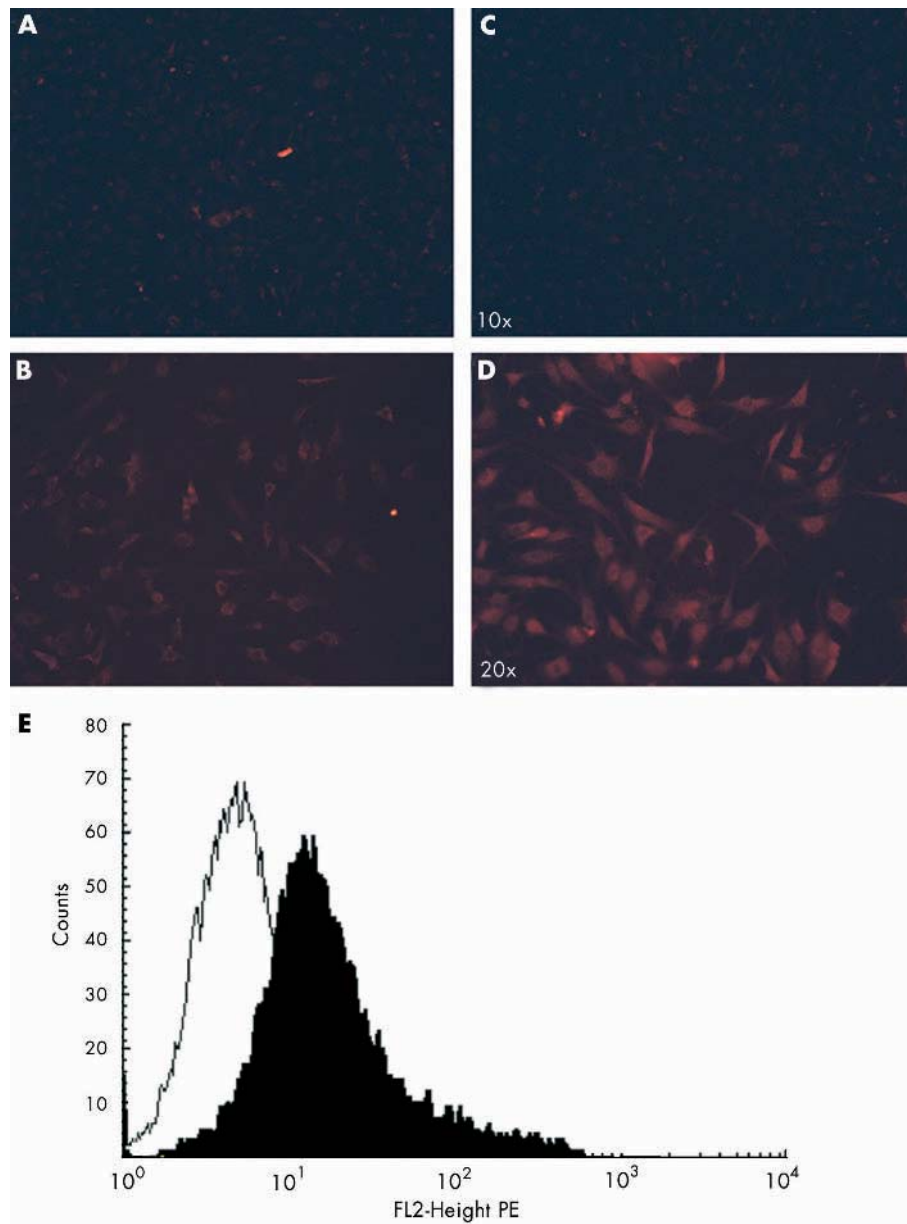


Figure 4 Immunofluorescence and flow cytometry detection of galectin-3 in OA chondrocytes. Cells were incubated with a specific anti-galectin-3 polyclonal antibody (B, D) or non-immune-serum (A, C) and detected with a fluorescent conjugated secondary antibody. (A, B) non-permeabilised cells; (C, D) permeabilised cells; (E) cell surface expression of galectin-3 by primary OA articular chondrocytes. Non-permeabilised cells were immunostained with a polyclonal anti-galectin-3 (filled histogram) or with non-immune serum (open histogram) followed by phycoerythrin (PE) conjugated secondary antibody. The assessment that chondrocytes were not permeabilised was performed by using the 7-AAD dye, which penetrates into the cells, only when the membrane is disrupted.

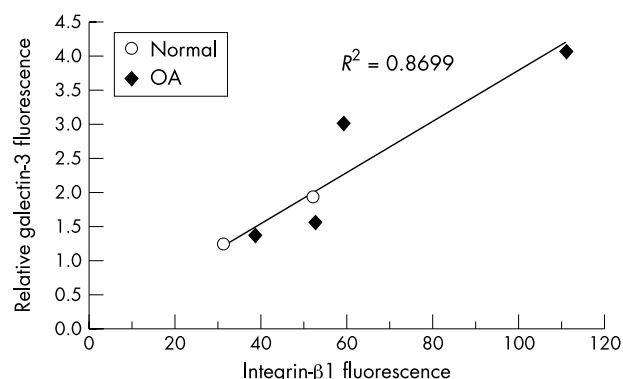


Figure 5 Correlation of galectin-3 presence with integrin-β1 at the chondrocyte surface. Cell surface fluorescence was measured with either galectin-3 serum or integrin-β1 antibody on non-permeabilised cells, as explained in "Materials and methods". As non-immune serum produced background level discrepancies, galectin-3 fluorescence was normalised to the non-immune signal. Relative galectin-3 fluorescence = galectin-3 fluorescence divided by non-immune fluorescence.

potential substrate for gelatinases A (MMP-2) and B (MMP-9). Interestingly, this domain has a high degree of homology with the type II collagen α1 chain.⁵³ Moreover, the amino acid site that might be recognised and cleaved by collagenase-3 is positioned three amino acids before the cleavage site recognised by gelatinases. As we and others have previously shown that among the collagenase family collagenase-3 (MMP-13) had the highest activity on type II collagen,^{42, 43} we further investigated whether galectin-3 might be a substrate for collagenase-3. Hence, we performed in vitro proteolysis experiments in which human recombinant galectin-3 in solution was found either in monomer or multimer form, as described by Ochieng *et al.*⁵⁴ We found that collagenase-3 cleaved galectin-3 whether the latter was present as a monomer or multimer (fig 6). In contrast, collagenase-1

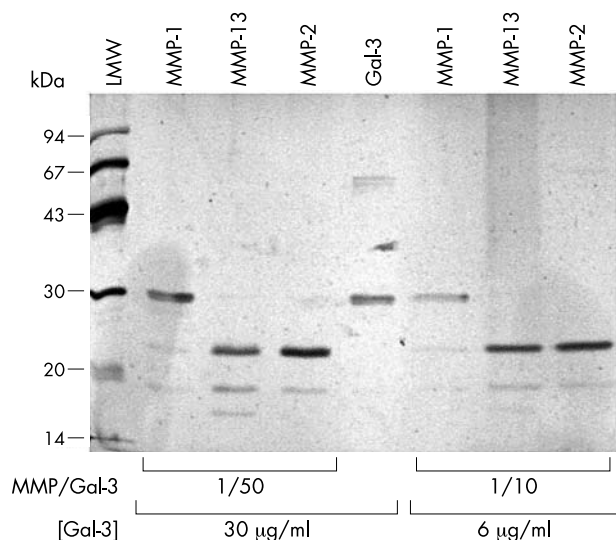


Figure 6 Proteolytic activity of collagenase-3 (MMP-13) on galectin-3. rh-galectin-3 was incubated in the presence of activated MMPs as described in "Materials and methods". As galectin-3 at high concentration could multimerise,²⁶ two galectin-3 concentrations were used: 30 μg/ml (multimerisation) and 6 μg/ml (monomerisation). The reaction mixture was stopped with a solution of 6×Laemmli buffer, loaded onto an SDS-PAGE system, and the galectin-3 was stained with silver nitrate. MMP-1, collagenase-1; MMP-13, collagenase-3; MMP-2, gelatinase A; Gal-3, galectin-3.

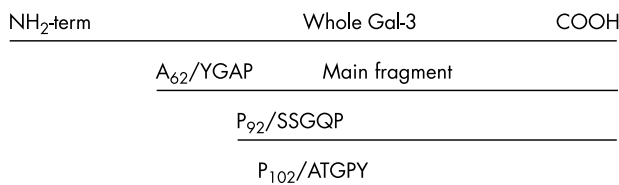


Figure 7 Schematic representation of galectin-3 sites generated by collagenase-3.

had very little activity on galectin-3. Gelatinase A was used as a positive control. Both the first and second fragments generated by collagenase-3 and gelatinase A had a similar molecular weight, about 22 and 18 kDa, respectively. However, as the potential collagenase-3 cleavage site is similar to the one found in type II collagen and is localised three amino acids before the gelatinase site, the discrimination between these two cleavage sites was performed by sequencing the first fragment. To this end, experimental conditions were chosen where galectin-3 could multimerise, thereby favouring collagenase activity. Sequencing analysis demonstrated that collagenase-3 cleaved galectin-3 at the identical site as the gelatinases (fig 7). Therefore, the main galectin-3 fragment from collagenase-3 was generated through its gelatinolytic activity.

DISCUSSION

Galectin-3 expression is strongly detected in pre- and hypertrophic chondrocytes before the signal is almost extinguished in late hypertrophic chondrocytes.^{28, 30} Its localisation at this stage of bone development is only intracellular. Moreover, its function was associated with the coordination of cell death and vascular invasion. OA chondrocytes are often compared with hypertrophic-like chondrocytes because they express hypertrophic markers normally found in the developing bone. This phenomenon could be associated with an attempt at cartilage remodelling in order to counteract the resorption occurring during the pathological process. However, this remodelling is incomplete or seems to be impaired by a lack of different factors normally present in the developing bone. A complementary explanation of these events could also be tied to a differential localisation of some proteins, which would then modify the relationship between chondrocytes and their ECM.

In this study we showed that galectin-3 expression was increased in OA cartilage and that this protein was expressed at the surface of adult chondrocytes in culture. This new localisation may correlate with a metabolic state of chondrocytes where galectin-3 acts as an interface between the ECM and the cells. Indeed, although speculative, it is possible that galectin-3 competes for different ligands localised either at the chondrocyte surface or in the ECM, leading to chondrocyte adhesion. When expressed to the cell surface from different cell types, galectin-3 was identified as a cell adhesion protein for laminin, type IV collagen, fibronectin, elastin, and tenascins,^{10, 15, 16, 55} and also as a receptor for advanced glycation end products.²³

Among the potential ligands cited above, published data showed that advanced glycation end products were increased both in the aging process and OA cartilage^{24, 25} and that OA chondrocytes produced more fibronectin,⁵⁶ which might therefore be present in the ECM. In response to the increasing amount of fibronectin, chondrocytes synthesised α5β1 integrin, the fibronectin receptor, to keep contact with the matrix. This is of importance, because adhesion regulates metabolic cell activity. Recently, Pulai *et al* showed that the chondrocyte survival signals were transmitted through the α5β1 integrin,²² while Kurtis *et al* demonstrated that this

integrin was implicated in chondrocyte adhesion.⁵⁷ This latter group also showed that blocking β_1 integrin subunits appeared to inhibit chondrocyte adhesion slightly more than did blocking of $\alpha_5\beta_1$ integrin, indicating that other integrins containing the β_1 subunit may be involved in chondrocyte adhesion to cartilage. Chondrocytes can also produce other integrins, such as $\alpha_1\beta_1$, $\alpha_3\beta_3$, $\alpha_6\beta_1$, $\alpha_4\beta_3$, and $\alpha_5\beta_5$,⁵⁸ but the α_1 and the β_1 integrin subunits are increased in OA,⁵¹ and in our study we have shown a strong correlation between galectin-3 and integrin- β_1 at the chondrocyte surface. Furthermore, it was recently shown that the $\alpha_1\beta_1$ integrin was a preferential receptor for type VI collagen,⁵⁸ the synthesis of which is increased in OA. Interestingly, Ochieng *et al* showed that both α_1 integrin, and β_1 integrin were ligands for galectin-3.¹⁵ Therefore, β_1 integrins may be targeted by galectin-3 during the OA process. Hence, the new localisation of galectin-3 in OA chondrocytes might play a part in the regulation of adhesive molecules towards the ECM. In fact, galectin-3 might interact with several ligands present either in the ECM or at the cell surface, and the affinity of galectin-3 for these ligands would lead to a regulation in the adhesion of chondrocytes and modification of internal signals. Parenthetically, although our number of specimens is low, it seems that macroscopic normal cartilage could contain some chondrocytes expressing a high level of integrin- β_1 at their surface, suggesting the beginning of the modification in the interaction of chondrocytes and their matrix.

Another point of regulation in the adhesiveness mediated by galectin-3 is the susceptibility of its N-terminal domain to cleavage by MMPs, in particular by MMP-2 and MMP-9.²⁶ The N-terminal domain allows galectin-3 multimerisation, but when this domain is cleaved, galectin-3 can no longer bridge two cells or a cell and the nearest ECM. Moreover, this proteolysis induces a modification in the affinity of galectin-3 for its ligands. As the N-terminal domain of galectin-3 was previously shown to share a high degree of homology with the type II collagen $\alpha 1$ chain, we investigated the collagenase-3 activity toward galectin-3. Indeed, collagenase-3 is the enzyme that has the highest activity on type II collagen among the members of the collagenase family. Our in vitro data showed that collagenase-3 cleaved galectin-3 at the identical site previously shown for MMP-2 and MMP-9 by others, indicating that the multimerisation of the N-terminal domain of galectin-3 did not re-create a triple helix as in the case of collagens. Therefore, the gelatinolytic activity of collagenase-3 was essential. This finding was interesting and might explain why collagenase-1 had almost no activity on galectin-3, because the gelatinolytic activity of collagenase-1 is 44-fold weaker than that of collagenase-3.⁵⁹ Moreover, collagenase-3 is increased during OA, particularly in the deep layer of the cartilage.⁴²⁻⁶⁰ Therefore, it can be suggested that galectin-3 and collagenase-3 might be involved in a coordinate process during cartilage remodelling.

In summary, this study showed that galectin-3 synthesis was increased in OA chondrocytes, and its localisation at the chondrocyte surface indicated a new function for these cells. Indeed, this study raises the hypothesis that galectin-3, when binding to different adhesive molecules and being cleaved by MMPs, may balance the adhesiveness of chondrocytes and this, more particularly, during the OA process.

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