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Stefan Toegel,* Daniela Weinmann,* Sabine André,[†] Sonja M. Walzer,* Martin Bilban,[‡] Sebastian Schmidt,[†] Catharina Chiari,* Reinhard Windhager,* Christoph Krall,[§] Idriss M. Bennani-Baiti,[¶] and Hans-Joachim Gabius[†]

Osteoarthritis is a degenerative joint disease that ranks among the leading causes of adult disability. Mechanisms underlying osteoarthritis pathogenesis are not yet fully elucidated, putting limits to current disease management and treatment. Based on the phenomenological evidence for dysregulation within the glycome of chondrocytes and the network of a family of adhesion/growth-regulatory lectins, that is, galectins, we tested the hypothesis that Galectin-1 is relevant for causing degeneration. Immunohistochemical analysis substantiated that Galectin-1 upregulation is associated with osteoarthritic cartilage and subchondral bone histopathology and severity of degeneration ($p < 0.0001$, $n = 29$ patients). In vitro, the lectin was secreted and it bound to osteoarthritic chondrocytes inhibitable by cognate sugar. Glycan-dependent Galectin-1 binding induced a set of disease markers, including matrix metalloproteinases and activated NF-κB, hereby switching on an inflammatory gene signature ($p < 10^{-16}$). Inhibition of distinct components of the NF-κB pathway using dedicated inhibitors led to dose-dependent impairment of Galectin-1–mediated transcriptional activation. Enhanced secretion of effectors of degeneration such as three matrix metalloproteinases underscores the data's pathophysiological relevance. This study thus identifies Galectin-1 as a master regulator of clinically relevant inflammatory-response genes, working via NF-κB. Because inflammation is critical to cartilage degeneration in osteoarthritis, this report reveals an intimate relation of glycobiology to osteoarthritic cartilage degeneration. *The Journal of Immunology*, 2016, 196: 1910–1921.

O steoarthritis, also known as osteoarthrosis or degenerative arthritis, is the most prevalent form of arthritis and a leading contributor to physical pain and disability, hereby shortening adult working life and causing considerable socioeconomic costs worldwide (1). The Center for Disease Control estimates osteoarthritis to afflict 13.9% of men and women aged 25–65 and 33.6% of those older than 65. More than 27 million Americans suffer from osteoarthritis, incurring a projected hospitalization cost of >$42 billion (2). Up to now, no cure for the progressive degeneration of joints in articular cartilage and subchondral bone, mostly seen in knees, hips, hands, and spine, has been identified. In addition to diminishing the joints' flexibility and shock-buffering capacity, the pain that accompanies osteoarthritis compromises the person's well-

being. Beyond direct coverage of all hospitalization-related expenses, osteoarthritis is therefore responsible for another $13 billion in lost productivity in the United States (2). Despite this enormously heavy human and economic toll, molecular mechanisms governing the pathogenesis of osteoarthritis are not yet fully understood. Advances in clarifying molecular causes would have the potential to markedly improve disease management and treatment. Due to the emerging significance of glycan-protein (lectin) recognition in diverse aspects of pathophysiology (3, 4), in this work we focus on a multifunctional lectin of the galectin family.

Galectins are potent regulators of adhesion and growth control via triggering outside-in signaling cascades after binding to distinct cell surface glycans, with relevance for inflammation (5–7). Obviously, coordinated changes in lectin and glycan presentation underlie the involvement of glycobiology in pathogenesis. With respect to osteoarthritis, we have characterized the human chondrocyte glycophenotype, showing that it was profoundly altered in patients suffering from cartilage degeneration (8, 9). Turning to galectins by immunohistochemical screening, initial evidence had suggested that Galectin-1 was associated to the level of degeneration in human osteoarthritic cartilage (10). This lectin is known to regulate cell-to-cell and cell-to-matrix communication via glycan bridging and subsequent signaling, acting as anti-inflammatory effector by inducing apoptosis of activated T cells (6). In relevant tumors, it is a marker useful for differential diagnosis between chondroblastic osteosarcoma and conventional chondrosarcoma (7, 11). In porcine articular chondrocytes, Galectin-1 modulates cellular interactions with constituents of the natural extracellular matrix as well as with an artificial matrix (12, 13), and, obviously relevant for degeneration, Galectin-1–dependent signaling upregulates >100-fold matrix

metalloproteinase (MMP)-13 gene transcription while at the same time downmodulating type II collagen (COL2A1) gene transcription (13). Reactivity to laminin, a well-known galectin counterreceptor in the extracellular matrix (14, 15), was documented for Galectin-1 in the intervertebral disc (16), and a positive Galectin-1 gradient from the proliferative to the hypertrophic zone was reported in growth-plate cartilage (13).

Work on clinical aspects of rheumatoid arthritis has uncovered a significant contribution of galectins to its pathogenesis (17, 18). In tissue specimens of these patients, Galectin-1 is mostly detected at the synovial sublining layer and downregulated in the synovial fluid (7, 19–22). Presence of specific autoantibodies, a common phenomenon for galectins in autoimmune diseases (23), may account for this decrease. Interestingly, the absence of Galectin-1 exacerbated disease manifestation in a mouse model of collagen-induced arthritis, in line with previous evidence of disease amelioration upon its delivery to sites of inflammation (24). Collectively, these data suggest that Galectin-1 may play a role in osteoarthritis pathogenesis and physiology, which, however, might be different from the protective (anti-inflammatory) activity in rheumatoid arthritis.

In this study, we first solidify and markedly extend the evidence for a significant correlation of Galectin-1 presence with the degree of osteoarthritic cartilage degeneration in patients, and then present data on Galectin-1 secretion, its lactose-inhibitable binding to chondrocytes, and definitive effector capacity upregulating disease markers, such as degradative enzymes on the mRNA and protein levels. Next, transcriptomic analyses for osteoarthritic chondrocytes disclose that Galectin-1 exerts control over a gene signature relevant for degeneration in osteoarthritis. We demonstrate that, upon cell surface binding in a lactose-inhibitable manner, Galectin-1 acts as master regulator of clinically relevant inflammatory-response genes centrally involving NF-κB, as unraveled by the systematic testing of a series of target-specific inhibitors.

Materials and Methods

Clinical specimens

Osteoarthritic cartilage was obtained from osteoarthritis patients undergoing total knee arthroplasty, whereas nonosteoarthritic cartilage specimens of the knee joint were collected postmortem from individuals without macroscopic signs of arthritic cartilage degeneration. Clinical specimens were obtained with written informed consent and in full accordance with the terms of the ethics committee of the Medical University of Vienna.

Histological assessment

Specimens of articular knee cartilage from 29 osteoarthritis patients (11 males, 18 females; 41–81 y) were selected macroscopically to cover a wide range of degeneration stages in each patient. Paraffin sections of mildly and severely degenerated cartilage were stained with Safranin O (Sigma-Aldrich) (10). Thereafter, a varying number of regions (2–10 per patient, depending on availability of regions differing in degeneration) was graded according to the Mankin score (MS), as previously described (10). In total, 231 regions were histologically graded prior to the processing of consecutive sections for immunohistochemical staining, as described below.

Immunohistochemistry

Immunohistochemical staining for Galectin-1 followed recently established protocols (10). Briefly, tissue sections were incubated with rabbit polyclonal Ab against human Galectin-1 (25). Ag-dependent staining was developed using 3,3′-diaminobenzidine tetrahydrochloride hydrate (FLUKA), whereas counterstaining was performed using Mayer's hemalum solution (Merck). The percentage of Galectin-1–positive chondrocytes in the regions of interest was determined independently by two observers, as described (10).

Production and quality control of recombinant Galectin-1 and FITC-labeled Galectin-1 (Galectin-1-FITC)

Human Galectin-1 was obtained by recombinant production and purified from bacterial extracts by affinity chromatography on lactosylated Sepharose 4B, prepared by ligand coupling to divinyl sulfone-activated

resin. Absence of contamination by bacterial LPS was ensured by a respective protocol (26), as purity was rigorously checked by one- and two-dimensional gel electrophoresis and mass-spectrometric fingerprinting, as previously reported (27). Fluorescent labeling under activity-preserving conditions was performed as described, and carbohydrate-binding activity was determined by cell-binding assays without or with cognate sugar used as an inhibitor (28).

Cell culture

Osteoarthritic and nonosteoarthritic chondrocytes were isolated from femoral condyles and tibial plateaus of articular cartilage following established protocols (8). Isolated chondrocytes were cultured in DMEM (Life Technologies) containing 10% FCS (Biochrom) and 100 μg/ml gentamicin (Life Technologies) in a humidified atmosphere of 5% CO_2/95% air at 37°C. To preserve the chondrocyte phenotype, only cells of passage 0 (osteoarthritic chondrocytes) and passage 1 (nonosteoarthritic chondrocytes) were used for assays.

Upon 90% confluency, chondrocyte cultures were starved overnight using DMEM supplemented with gentamicin and treated with recombinant Galectin-1—in the presence or absence of the cognate sugar lactose (Sigma-Aldrich) to block binding to glycans—in starvation medium for the indicated time periods. For inhibition of NF-κB pathway components, inhibitors acting at distinct sites (Calbiochem; please see Fig. 7 for details on their molecular targets and used concentrations) were added to the chondrocyte cultures 1 h prior to the stimulation with recombinant Galectin-1. Additionally, chondrocyte cultures were stimulated with IL-1β, TNF-α, or IL-8 (all from BioLegend) to induce proinflammatory conditions in vitro.

Immunofluorescent detection of Galectin-1 binding sites

A 50 μl cell suspension (3×10^5 cells, prepared by trypsinization of monolayer cultures) was incubated in presence and absence of 0.1 M lactose together with 50 μl PBS containing 10 μg Galectin-1-FITC for 10 min at 4°C, limiting internalization of cell-bound Galectin-1. After removing any unbound lectin by washing with PBS, cells were immediately mounted for microscopy without fixation. Confocal images of fluorescence-labeled cells were obtained using a Carl Zeiss LSM 700 Laser Scanning Microscope at original magnification ×63 and Zen software.

Quantitative RT-PCR

Isolation of total RNA, cDNA synthesis, and SYBR Green–based quantitative RT-PCR (RT-qPCR) experiments followed established protocols (29, 30). Briefly, total RNA was extracted from Galectin-1–treated osteoarthritic chondrocytes and examined for quality and quantity on the Agilent 2100 Bioanalyzer Nano LabChip (Agilent Technologies), prior to reverse transcription into cDNA. The protocols thoroughly followed the minimal guidelines for the design and documentation of quantitative PCR experiments, as recently outlined (31). A detailed quantitative PCR checklist is readily provided by the authors upon request.

ELISA

The levels of ADAMTS-4 (BosterBio), Galectin-1, proMMP-1, total MMP-3, and proMMP-13 (all from R&D Systems), secreted into the cell culture medium by Galectin-1–treated osteoarthritic chondrocytes, were determined by commercial ELISAs, according to the manufacturers' protocols. ELISA standard curve ranges were 0.625–40 ng/ml in the case of ADAMTS-4, 0.313–20 ng/mg in the case of Galectin-1, 0.156–10 ng/ml in the cases of proMMP-1 and total MMP-3, and 78–5000 pg/ml in the case of proMMP-13. The lowest concentration that could be reliably determined was set as the detection limit.

Microarray

Osteoarthritic chondrocytes were isolated from five male patients (47–78 y). Following starvation, cells were incubated in the presence of 50 μg/ml recombinant Galectin-1 for 24 h. Total RNA was extracted and extensively analyzed for quantity, purity, and integrity using the Agilent 2100 Bioanalyzer and the Nanodrop 1000. RNA integrity numbers were between 9.1 and 10, and A260/A280 values were between 2.02 and 2.11. GeneChip analysis was performed using 200 ng total RNA per sample. Preparation of terminally labeled cRNA, hybridization to genome-wide human PrimeView GeneChips (Affymetrix), and scanning of the arrays were carried out according to the manufacturer's protocols. Robust multichip average signal extraction, normalization, and filtering were performed as described using the comprehensive R- and bioconductor-based web service for microarray data analysis CARMAweb (32). A variation filter was applied for selecting informative (i.e., significantly varying) genes. The filtering criteria for the

exemplary data sets required an interquartile range of >0.5 and at least one sample with expression intensity of >100. The microarray data discussed in this publication have been deposited in the Gene Expression Omnibus database and are publicly accessible through Gene Expression Omnibus Series accession number GSE68760 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=snqzskeenhgznef&acc=GSE68760).

Bioinformatic analyses

Quantification of differential gene expression was carried out using a moderated two-sample *t* test, as previously described (33). The gene set enrichment analyses were performed, as previously reported (34). Briefly, gene set enrichment analyses studies were conducted in the Molecular Signatures Database, and the MSigDB C2, C3, and C5 collections were searched for significant correlations. Overrepresentation of a given transcription factor binding site (TFBS) in any given gene set was ascertained by comparing the TFBS occurrence in the gene set with its occurrence in the human genome and in all known human promoters. The *p* values were obtained by hypergeometric testing and were further corrected for multiple testing using the Benjamini-Hochberg method. The threshold to consider *p* values as significant was 0.05. Promoter analysis for identification of putative binding sites for transcription factors was carried out, as described (35).

Genes studied

To avoid gene homonymy or species ambiguity (36), genes discussed in this work are listed below with their unique National Center for Biotechnology Information GeneID: *ADAMTS4* (GeneID: 9507), *AGC1* (176), *CCL4* (6351), *CCL5* (6352), *COL2A1* (1280), *CXCL1* (2919), *CXCL2* (2920), *CXCL3* (2921), *CXCL5* (6374), *FRZB* (2487), *GREM1* (26585), *IL1B* (3553), *IL6* (3569), *IL8* (3576), *IRAK* (3654), *LGALS1* (3956), *MMP1* (4312), *MMP3* (4314), *MMP13* (4322), *NFKB1* (4790), *RELA* (5970), *SELE* (6401), *ST3GAL1* (6482), *ST6GAL1* (6480), and *TNFA* (7124).

Western blot

Proteins were extracted from chondrocytes after treatment with recombinant Galectin-1, as indicated. Cells were thoroughly washed with PBS and lysed with radioimmunoprecipitation assay lysis buffer (Santa Cruz), supplemented with PMSF, sodium orthovanadate, and a protease inhibitor mixture, for 1 h on ice. The cell lysate was then further processed in an ice-cold ultrasonic bath, thoroughly vortexed, and centrifuged. Proteins in the supernatants were separated using 5% acrylamide stacking and 10% acrylamide separating gels and transferred to a nitrocellulose membrane by electroblotting (Protran; Schleicher & Schuell). Binding sites for proteins on the membrane were blocked overnight at 4°C with PBS (Life Technologies) containing 5% milk powder (Roth). Membranes were incubated for 2 h with anti–IκBα (N-terminal Ag, 1:1,000, mouse monoclonal; Cell Signaling), anti–phospho-IκBα (Ser$^{32/36}$, 1:1,000, mouse monoclonal; Cell Signaling), anti–NF-κB p65 (1:1,000, rabbit monoclonal; Cell Signaling), or anti–phospho–NF-κB p65 (Ser^{536}, 1:1,000, rabbit monoclonal; Cell Signaling) together with anti–β-actin (1:5,000, mouse monoclonal; Sigma-Aldrich) in Odyssey Blocking Buffer (LI-COR Biosciences), which had been diluted with PBS containing 0.1% Tween 20 (Bio-Rad). Thereafter, the membrane was incubated for 1 h with DyLight 800 nm-labeled goat anti-rabbit IgG and IRDye 680LT goat anti-mouse IgG, both diluted 1:15,000 in PBS containing 0.1% Tween 20. Finally, the immunoreactive protein bands were detected and quantified using the Odyssey Imager with Image Studio Software (LI-COR Biosciences). The ratio between p-IκBα and IκBα or between p-p65 and p65 (all normalized for β-actin) was expressed as relative quantity in comparison with the untreated control set to 1.

Statistical analyses

Statistical analysis of a correlation between Galectin-1 immunopositivity and the MS was performed using SAS 4.3. Pearson's correlation coefficients were calculated for each patient separately, and the Wilcoxon signed-rank test was performed to test whether the median correlation coefficient was different from 0. Statistical comparison of the microarray data was performed using the *limma* algorithm (32), and multiple testing correction based on the false discovery rate was performed to produce adjusted *p* values. Statistical analyses of RT-qPCR, ELISA, and quantitative Western blot data were performed using SPSS 20.0. Normality of the data was analyzed using the Kolmogorov-Smirnov test, and statistical significance of the data was delineated using paired or unpaired *t* tests or Wilcoxon signed-rank test. All analysis units (*n*) given in figure and table legends—unless otherwise stated—refer to the number of independent observations (biological replicates) underlying the respective descriptive statistics and statistical tests.

Results

Presence of Galectin-1 in articular chondrocytes correlates with osteoarthritis-related cartilage degeneration

To test the hypothesis for a role of Galectin-1 as bioeffector of osteoarthritis, we first assessed in detail the immunopositivity of chondrocytes for Galectin-1 in relation to the degeneration status of osteoarthritic cartilage in 29 patients. Fig. 1A shows three prototypical cartilage regions of a representative patient, as follows: one region with almost intact surface and tissue morphology (MS1);
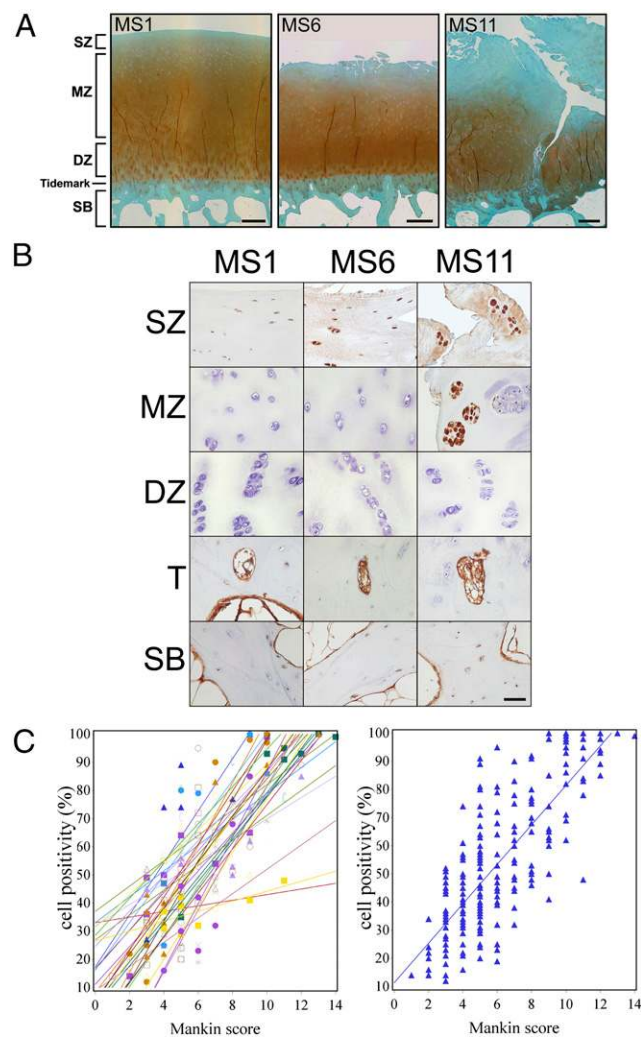
FIGURE 1. Localization of Galectin-1 in human osteoarthritic knee cartilage and correlation of chondrocyte positivity with cartilage degeneration. (**A**) Histological sections of articular cartilage/subchondral bone (*n* = 29 patients) were stained with Safranin O and categorized according to their degeneration status using the MS. Shown is an exemplary series from one representative donor presenting mildly (MS1), moderately (MS6), and severely (MS11) degenerated regions. Overview images were photomerged using Adobe Photoshop from single photographs. Scale bars, 500 μm. (**B**) Immunohistochemistry of the articular cartilage sections shown in (A), using an Ab against Galectin-1 without cross-reactivity to other human galectins. Scale bar, 50 μm. (**C**) From each of the 29 patients, 2–10 (8 ± 1.7) regions were histologically graded according to the MS and consecutive sections were stained immunohistochemically for Galectin-1. The percentage of Galectin-1–positive chondrocytes was assessed in each region. Shown are both the scatterplots of Galectin-1 positivity versus MS together with the regression lines for each patient (*left panel*) and with the regression line over all patients (*right panel*). DZ, deep zone; MZ, middle zone; SB, subchondral bone; SZ, superficial zone; T, tidemark region.

one with considerable surface discontinuity and matrix depletion (MS6); and one with vertical clefts, matrix loss, formation of chondrocyte clusters, and blood vessels penetrating the tidemark (MS11). Immunohistochemical analysis of respective consecutive sections revealed that the presence of Galectin-1 was restricted to blood vessels and a subset of subchondral trabecular osteocytes in the MS1 region (Fig. 1B). In full agreement with previous findings in nonosteoarthritic cartilage from tumor patients (10), articular chondrocytes were immunonegative from the superficial to the deep zone in this intact cartilage region. In the MS6 region, by contrast, chondrocytes of the superficial zone were predominantly positive for Galectin-1, whereas no positivity was found in chondrocytes of the middle and deep zone. Most interestingly, we observed strong Galectin-1–dependent staining in all chondrocytes in the superficial zone of the MS11 region, together with focal staining in chondrocytes in the middle zone. As depicted in Fig. 1B, the presence of Galectin-1 in middle-zone chondrocytes was preferentially localized near deep vertical clefts or fissures. Likewise, chondrocytes assembled in clusters were always positive for Galectin-1, even in the absence of vertical clefts (data not shown).

From these data, extending our initial previous findings (10), we reasoned that the presence of Galectin-1 in osteoarthritic chondrocytes is intimately connected with osteoarthritis severity and associated with histopathologic features of osteoarthritic cartilage and subchondral bone. Therefore, we determined the percentage of Galectin-1–positive chondrocytes in each region of the 29 test series and performed correlation analyses between Galectin-1 immunopositivity and the MS for each patient (Fig. 1C). Statistical analysis disclosed a strong correlation between Galectin-1 levels and degeneration severity ($p < 0.0001$). Sex-specific stratification of data excluded any significant difference between female and male patients (data not shown).

Taken together, these results demonstrate that Galectin-1 accumulates in articular osteoarthritic chondrocytes of the superficial/middle zones and that the percentage of Galectin-1–positive chondrocytes correlates with increasing degeneration status of cartilage. As next step, we explored whether chondrocytes have the capacity to secrete Galectin-1 and whether expression and secretion of Galectin-1 might be induced by early signals of proinflammatory conditions.

Secretion of Galectin-1 by isolated chondrocytes and lack of regulatory response to proinflammatory cytokines

As shown in Fig. 2A, isolated osteoarthritic chondrocytes secreted 1.65 ± 2.27 ng/ml Galectin-1 into the supernatant within 24 h of monolayer culture ($n = 4$ patients). Presence of the proinflammatory cytokines IL-1β, TNF-α, or IL-8 did not significantly alter the amount of secreted Galectin-1 (Fig. 2A; $p > 0.05$). This finding was extended by RT-qPCR analysis of *LGALS1* mRNA to gene expression levels in osteoarthritic chondrocytes (Fig. 2B) and nonosteoarthritic chondrocytes (Fig. 2C). The data indicate that the expression and secretion of Galectin-1 by isolated chondrocytes did not appear to be sensitive to proinflammatory conditions. Of note, the same concentration of TNF-α caused a strong signal enhancement for Galectin-1 in Western blots of extracts from monocyte-dependent PBMCs, being as effective as stimulation by anti-CD3 (37). This underscores the need to consider the cell type when studying the induction of Galectin-1.

To act as an effector, extracellular Galectin-1 needs to bind to osteoarthritic chondrocytes. We thus next investigated Galectin-1–chondrocyte cell surface binding using Galectin-1-FITC.

Galectin-1 binds to osteoarthritic chondrocytes via glycans and affects the expression of functional osteoarthritis markers

Chondrocytes labeled with Galectin-1-FITC exhibited fluorescence staining of cell membranes (Fig. 2D). As documented,
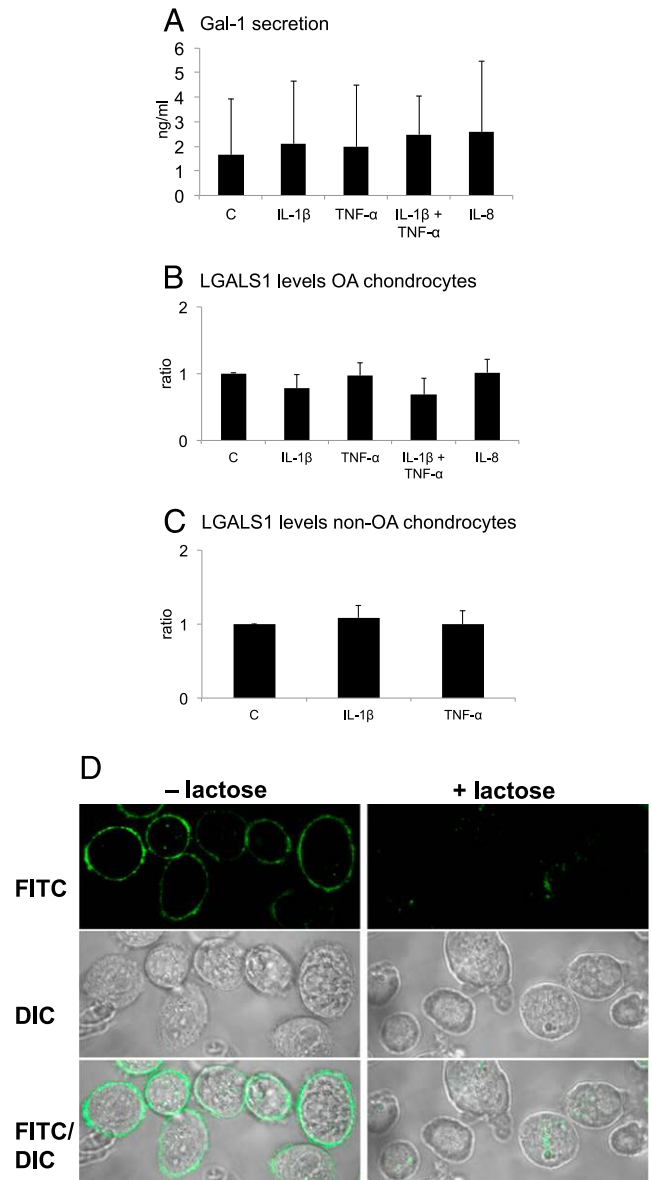

FIGURE 2. Galectin-1 is expressed and secreted by cultured chondrocytes and binds to cell surfaces via glycans. (**A**) Osteoarthritic chondrocytes ($n = 4$ patients) were starved overnight prior to treatment with 10 ng/ml IL-1β, 10 ng/ml TNF-α, a combination of 10 ng/ml IL-1β and 10 ng/ml TNF-α, or 100 ng/ml IL-8 for 24 h and collection of cell culture supernatants. Secreted Galectin-1 levels (ng/ml) were determined using ELISA. Results are shown as mean ± SD. (**B**) Osteoarthritic chondrocytes ($n = 4$ patients) were starved overnight prior to treatment with 10 ng/ml IL-1β, 10 ng/ml TNF-α, a combination of 10 ng/ml IL-1β and 10 ng/ml TNF-α, or 100 ng/ml IL-8 for 24 h and isolation of total RNA. *LGALS1* mRNA levels were determined using RT-qPCR. Results are expressed as fold changes (mean ± SD) versus untreated controls set to 1. (**C**) Nonosteoarthritic chondrocytes ($n = 3$ individuals) were starved overnight prior to treatment with 10 ng/ml IL-1β or 10 ng/ml TNF-α for 24 h and isolation of total RNA. *LGALS1* mRNA levels were determined using RT-qPCR. Results are expressed as fold changes (mean ± SD) versus untreated controls set to 1. (**D**) Cultured osteoarthritic chondrocytes were trypsinized and resuspended prior to labeling with Galectin-1-FITC (green) at 4°C in the absence or presence of 0.1 M lactose. After 10 min of incubation, cells were washed and analyzed using laser-scanning microscopy, with the focus plane set to the center of cells. Fluorescence images as well as the corresponding differential interference contrast images and overlay images are presented. Shown are the results from chondrocytes of one patient, representative for three independent experiments ($n = 3$ patients).

addition of cognate sugar (lactose) precluded binding of Galectin-1 to the surface of chondrocytes. This evidence for glycan-dependent binding intimated that Galectin-1 may trigger an outside-in signaling by glycoconjugate cross-linking (lattice formation). This process may modulate chondrocyte gene expression.

Thus, we next measured the effects of Galectin-1 on functional marker genes in cultured human chondrocytes with LPS-free Galectin-1 (26, 27). *ADAMTS4* (Fig. 3A), *MMP1* (Fig. 3B), *MMP3* (Fig. 3C), and *MMP13* (Fig. 3D) mRNA levels were found to be significantly upregulated by Galectin-1 in a concentration-dependent manner. Interestingly, Galectin-1 sig-
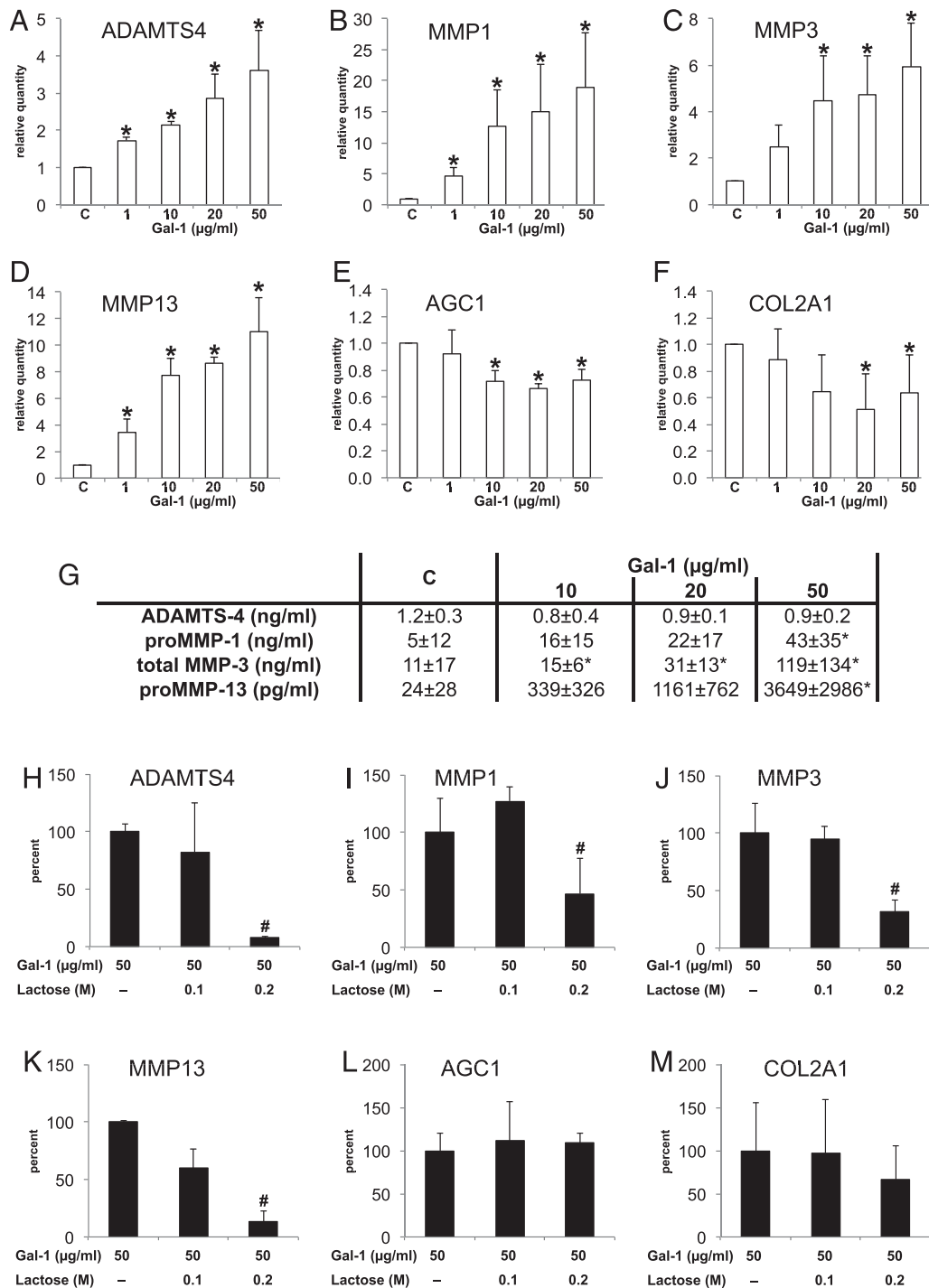
FIGURE 3. Galectin-1 affects the expression of genes for matrix-related proteins and enzymes via binding to glycans. (**A–G**) Osteoarthritic chondrocytes ($n = 5$ patients) were starved overnight prior to treatment with 1–50 μg/ml Galectin-1 for 24 h and isolation of total RNA and collection of supernatants. RT-qPCR was performed for (A) *ADAMTS4*, (B) *MMP1*, (C) *MMP3*, (D) *MMP13*, (E) *AGC1*, and (F) *COL2A1* using validated assays. Results are expressed as fold changes (mean ± SD) versus untreated controls set to 1. *$p < 0.05$ (paired t test). (G) Cell culture supernatants were monitored by ELISA for ADAMTS-4 (ng/ml), proMMP-1 (ng/ml), total MMP-3 (ng/ml), and proMMP-13 (pg/ml); *$p < 0.05$ (paired t test). (**H–M**) Osteoarthritic chondrocytes ($n = 3$ patients) were starved overnight prior to treatment with 50 μg/ml Galectin-1 for 24 h in presence or absence of 0.1 or 0.2 M β-lactose. mRNA levels of (H) *ADAMTS4*, (I) *MMP1*, (J) *MMP3*, (K) *MMP13*, (L) *AGC1*, and (M) *COL2A1* were assessed using RT-qPCR and expressed as percentage (mean ± SD) of Galectin-1–treated chondrocytes. #$p < 0.05$ (paired t test versus Galectin-1–treated chondrocytes).

FIGURE 4. The 20 most up- and downregulated genes in osteoarthritic chondrocytes using Galectin-1 as effector. (**A**) Osteoarthritic chondrocytes (*n* = 5 patients; numbered with "1–5") were starved overnight prior to treatment with 50 µg/ml Galectin-1 for 24 h. Following microarray analysis, heat maps of the 20 most upregulated genes and the 20 most downregulated genes were plotted. (**B**) For these genes, the ratio between mRN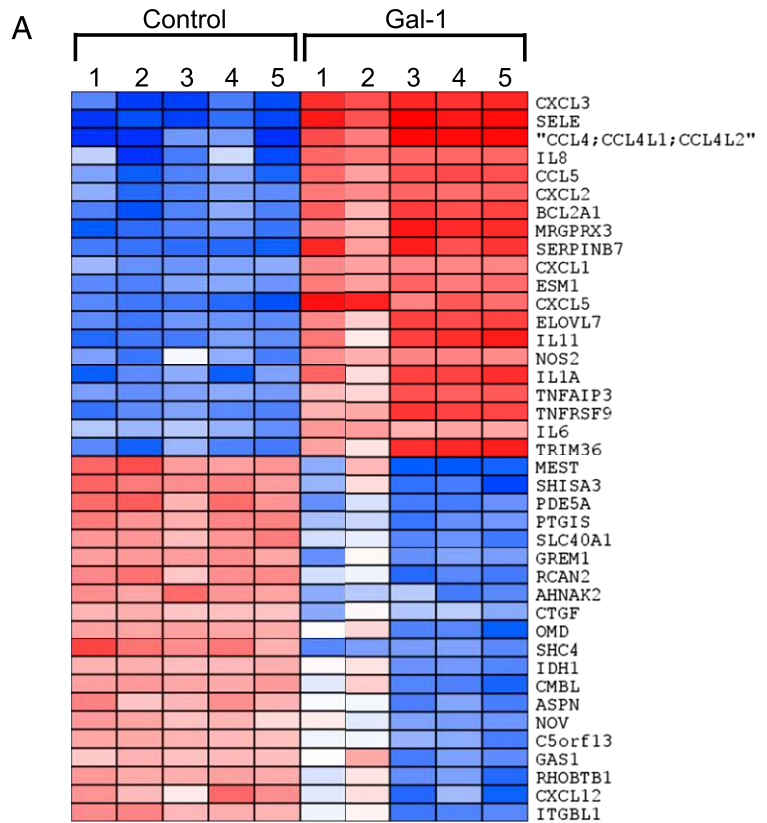A levels in Galectin-1–treated versus untreated chondrocytes across all five patients was calculated. The *p* values, corrected for multiple hypothesis testing by the Benjamini-Hochberg method (BH), are also given. (**C**) The results of the microarray experiments were ascertained using RT-qPCR in the same cDNA samples as used in the microarray analysis. Values are given as fold changes with respect to untreated controls set to 1. Means and SDs were computed, normality of the data was analyzed using Kolmogorov-Smirnov (KS) test, and statistical significance of the data was examined using paired *t* test and Wilcoxon signed-rank test.

B

Symbol	Entrez ID	Gene name	BH	Ratio treated/untreated
CXCL3	2921	chemokine (C-X-C motif) ligand 3	4E-08	424.0
SELE	6401	selectin E	3E-07	323.3
CCL4;CCL4L1;CCL4L2	388372;6351;9560	chemokine (C-C motif) ligand 4 /// chemokine (C-C motif) ligand 4-like 1 /// chemokine (C-C motif) ligand 4-like 2	3E-05	273.0
IL8	3576	interleukin 8	3E-05	230.4
CCL5	6352	chemokine (C-C motif) ligand 5	7E-06	181.4
CXCL2	2920	chemokine (C-X-C motif) ligand 2	2E-07	141.8
BCL2A1	597	BCL2-related protein A1	3E-05	120.6
MRGPRX3	117195	MAS-related GPR, member X3	1E-04	104.6
SERPINB7	8710	serpin peptidase inhibitor, clade B (ovalbumin), member 7	2E-05	97.5
CXCL1	2919	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	7E-08	97.1
ESM1	11082	endothelial cell-specific molecule 1	7E-07	87.6
CXCL5	6374	chemokine (C-X-C motif) ligand 5	2E-05	75.9
ELOVL7	79993	ELOVL family member 7, elongation of long chain fatty acids (yeast)	1E-04	75.9
IL11	3589	interleukin 11	4E-04	70.6
NOS2	4843	nitric oxide synthase 2, inducible	7E-05	69.6
IL1A	3552	interleukin 1, alpha	2E-04	60.7
TNFAIP3	7128	tumor necrosis factor, alpha-induced protein 3	3E-04	56.4
TNFRSF9	3604	tumor necrosis factor receptor superfamily, member 9	1E-04	54.4
IL6	3569	interleukin 6 (interferon, beta 2)	1E-06	52.8
TRIM36	55521	tripartite motif-containing 36	8E-04	52.0

Symbol	Entrez ID	Gene name	BH	Ratio treated/untreated
MEST	4232	mesoderm specific transcript homolog (mouse)	2E-03	0.027
SHISA3	152573	shisa homolog 3 (Xenopus laevis)	5E-04	0.032
PDE5A	8654	phosphodiesterase 5A, cGMP-specific	7E-05	0.036
PTGIS	5740	prostaglandin I2 (prostacyclin) synthase	2E-05	0.036
SLC40A1	30061	solute carrier family 40 (iron-regulated transporter), member 1	2E-04	0.039
GREM1	26585	gremlin 1	1E-04	0.041
RCAN2	10231	regulator of calcineurin 2	3E-04	0.052
AHNAK2	113146	AHNAK nucleoprotein 2	3E-05	0.052
CTGF	1490	connective tissue growth factor	3E-04	0.061
OMD	4958	osteomodulin	3E-03	0.068
SHC4	399694	SHC (Src homology 2 domain containing) family, member 4	4E-05	0.074
IDH1	3417	isocitrate dehydrogenase 1 (NADP+), soluble	3E-03	0.077
CMBL	134147	carboxymethylenebutenolidase homolog (Pseudomonas)	2E-03	0.077
ASPN	54829	asporin	1E-03	0.078
NOV	4856	nephroblastoma overexpressed gene	2E-03	0.083
C5orf13	9315	chromosome 5 open reading frame 13	6E-04	0.086
GAS1	2619	growth arrest-specific 1	2E-02	0.088
RHOBTB1	9886	Rho-related BTB domain containing 1	1E-03	0.088
CXCL12	6387	chemokine (C-X-C motif) ligand 12	5E-03	0.088
ITGBL1	9358	integrin, beta-like 1 (with EGF-like repeat domains)	1E-03	0.092

C

Donor	Control	IL8	CXCL1	CXCL2	CXCL3	CXCL5	IL6	SELE	GREM1	FRZB	ST6Gal1	ST3Gal1
1	1	642	223	154	421	271	213	658	0.23	0.82	0.43	3
2	1	447	173	96	287	51	83	51	0.13	0.43	0.37	2
3	1	4332	2786	1738	2119	125	34	4011	0.48	0.51	0.08	10
4	1	1612	698	623	890	157	120	692	0.37	0.43	0.16	11
5	1	3313	1711	1392	2024	152	168	1184	0.66	0.57	0.13	8
Mean		2069	1118	801	1148	151	124	1319	0.37	0.55	0.23	7
S.D.		1699	1118	737	873	79	70	1558	0.21	0.16	0.16	4
KS-test		0.200	0.200	0.200	0.200	0.200	0.200	0.070	0.200	0.200	0.200	0.200
paired t-test		0.026	0.045	0.036	0.021	0.007	0.009	-	0.001	0.002	0.000	0.018
Wilcoxon		0.031	0.031	0.031	0.031	0.031	0.031	0.031	0.031	0.031	0.031	0.031

nificantly and concentration dependently reduced *COL2A1* and *AGC1* mRNA expression (Fig. 3E, 3F). At the protein level, Galectin-1–treated chondrocytes secreted large amounts of proMMP-1, MMP-3, and proMMP-13 ($p < 0.05$; Fig. 3G), again in a concentration-dependent manner, whereas ADAMTS-4 protein levels were not significantly modified by Galectin-1. In agreement with inhibition of Galectin-1 binding to chondrocytes in the presence of lactose (Fig. 2), effects of Galectin-1 on mRNA levels were dose dependently reduced to basal levels by lactose in the cases of *ADAMTS4*, *MMP1*, *MMP3*, and *MMP13* (Fig. 3H, 3I, respectively), also confirming the lack of LPS contamination in the Galectin-1 preparation used. In contrast, however, the modest Galectin-1–induced reduction of mRNA expression *COL2A1* and *AGC1* was not significantly reversed by lactose (Fig. 3J, 3K).

Together, these findings indicated that, by binding to chondrocytes in a lactose-inhibitable manner, Galectin-1 upregulates presence of functional disease markers involved in matrix degradation. To take this activity to the genome level and characterize the route to these disease-associated effects, we carried out genome-wide and molecular analyses, to uncover the capacity of Galectin-1 to differentially modulate genes with relevance to osteoarthritis pathogenesis.

Galectin-1 establishes a chondrocyte mRNA signature associated with inflammation and NF-κB signaling

Indeed, genome-wide analysis revealed a set of differentially regulated genes of particular interest. Among the most upregulated genes in the presence of Galectin-1, a group of genes for chemokine ligands, including *CXCL3*, *CCL4*, *CCL5*, *CXCL2*, *CXCL1*, and *CXCL5*, together with other genes such as *SELE* and *IL8*, was found (Fig. 4A, 4B). On the other side of the spectrum, bone morphogenetic protein antagonist *GREM1* was among the most downregulated genes. RT-qPCR experiments confirmed that *IL8*, *IL6*, *SELE*, and *ST3GAL1* were strongly upregulated, whereas *GREM1*, *FRZB*, and *ST6GAL1* (an enzyme introducing a block to Galectin-1 binding of N-glycans) were significantly downregulated by Galectin-1 (Fig. 4C). Because some genes are highly sensitive to gene dosage, we also list in Supplemental Table I moderately modulated genes, including Galectin-1–regulated glycogenes (enzymes and lectins) and osteoarthritis-related genes. These genes may also contribute to disease progression alone or in combination and may thus be worth pursuing in future studies.

The genome-wide analyses showed that treatment of primary human chondrocytes with Galectin-1 induces an inflammation gene signature with very high significance ($p < 10^{-16}$). The Galectin-

FIGURE 5. Bioinformatic investigation of genes induced in chondrocytes treated with Galectin-1. Data shown are Venn diagram representations. The size of circles is indicative of the gene set size, and the size of intersections, also indicated with a number in bold, depicts the number of shared genes between the two gene sets. (**A**) C2 analysis of perturbation signatures associated with Galectin-1 treatment: the 12 most significant signatures are listed from the most (1) to least significant (12) based on the extent of gene overlap between the chondrocytes/Galectin-1 gene set and paired gene signatures. (**B**) C2 analysis of canonical and KEGG pathways associated with Galectin-1 treatment: the eight most significant signatures are ranked from the most (1) to least significant (8). (**C**) C2 analysis of reactome pathways associated with Galectin-1 treatment: the top four signatures are listed by decreasing significance from 1 to 4.
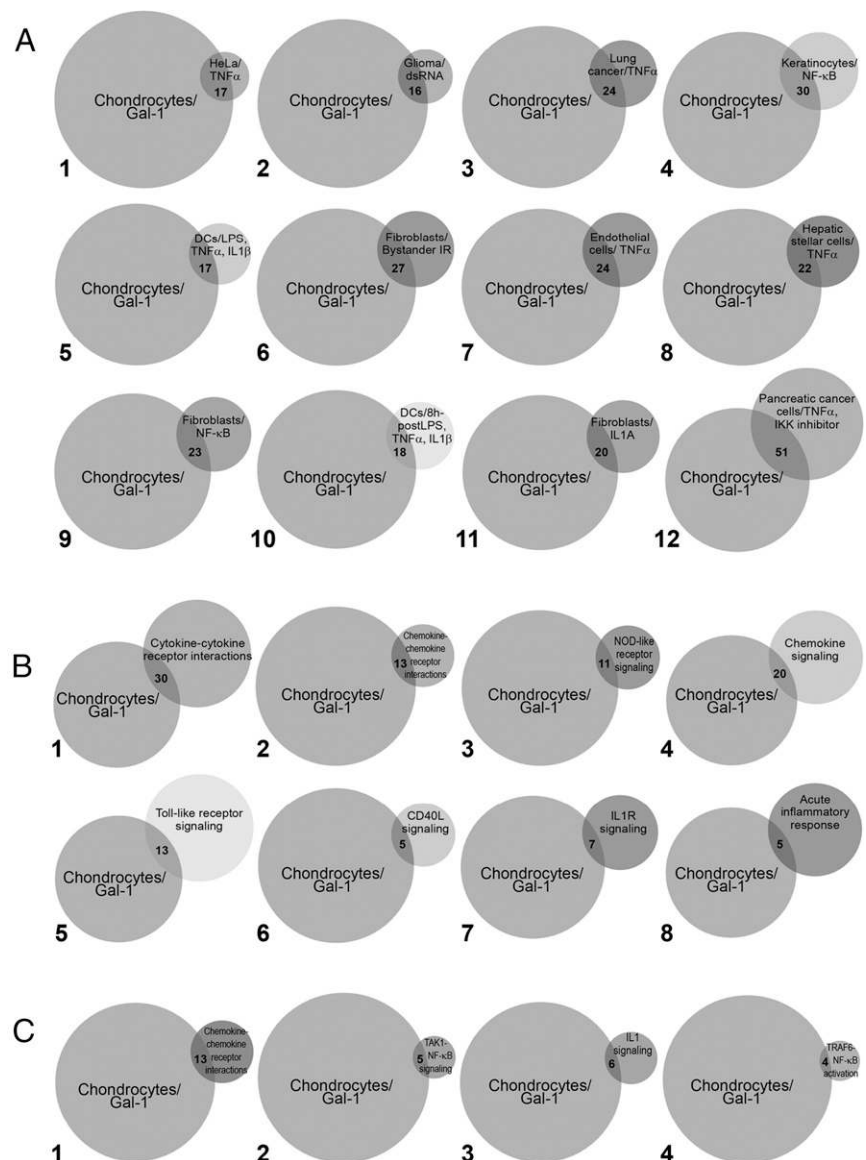
Table I. C5-GSEA analysis of Galectin-1–treated chondrocytes

Gene Set Name	Description	Genes in Overlap (k)	Genes in Gene Set (K)	k/K	p Value
Inflammatory response	Genes annotated by the GO term GO:0006954. The immediate defensive reaction (by vertebrate tissue) to infection or injury caused by chemical or physical agents. The process is characterized by local vasodilation, extravasation of plasma into intercellular spaces, and accumulation of WBCs and macrophages.	21	129	0.1628	1.87×10^{-6}
Response to wounding	Genes annotated by the GO term GO:0009611. A change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a stimulus indicating damage to the organism.	25	190	0.1316	1.06×10^{-5}
Response to extrastimulus	Genes annotated by the GO term GO:0009605. A change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of an external stimulus.	29	312	0.0929	1.17×10^{-3}
Defense response	Genes annotated by the GO term GO:0006952. Reactions, triggered in response to the presence of a foreign body or the occurrence of an injury, which result in restriction of damage to the organism attacked or prevention/recovery from the infection caused by the attack.	26	270	0.0963	1.26×10^{-3}
Locomotory behavior	Genes annotated by the GO term GO:0007626. The specific movement from place to place of an organism in response to external or internal stimuli. Locomotion of a whole organism in a manner dependent upon some combination of that organism's internal state and external conditions.	11	95	0.1158	8.77×10^{-3}

K, number of genes included in biological processes; k, number of overlapping genes induced by Galectin-1.

1–imparted inflammation signature showed up in three different types of analyses, including those of reactome pathways, chemical and genetic perturbation signatures, and canonical and KEGG signaling pathways (Fig. 5). An additional analysis of signaling molecules downstream of Galectin-1 reveals a major overlap with several biological processes (e.g., response to wound or defense mechanisms), wherein inflammation is a core component (Table I). Analysis of TFBS overrepresented in promoters of genes selectively upregulated following induction by Galectin-1 revealed strong representation of an NF-κB (and NF-κB subunits RELA and NFKB1; $p < 5.9 \times 10^{-3}$– 1.55×10^{-6}) signature, suggesting that the Galectin-1–induced switch to a prodegenerative expression program in human osteoarthritic chondrocytes largely involves NF-κB (Table II).

The activity of Galectin-1 in osteoarthritic chondrocytes is mediated via the NF-κB pathway

Based on the bioinformatic findings, we next sought to biochemically test the role of NF-κB in the signal transduction triggered by Galectin-1. Therefore, the direct impact of Galectin-1 on the activation of the canonical NF-κB pathway was assessed over time (15 min–16 h). As presented in Fig. 6A (*first lane*), Galectin-1 significantly induced the phosphorylation of IκBα in a time-dependent manner, with a peak at 1 h after starting of the treatment and subsequent reduction to basal levels. Taking the levels of IκBα into account (Fig. 6A, *second lane*), the ratio of p-IκBα/IκBα strongly and time dependently increased at 1 and 4 h (Fig. 6B; $n = 3$ patients; $p < 0.05$), pointing to phosphorylation and proteolytic degradation of IκBα as a result of exposure to Galectin-

Table II. C3-GSEA analysis of Galectin-1–treated chondrocytes

Transcription Factor	Transcription Factor Binding Motif	k/K	p Value
c-rel	SGGRNTTTCC	0.0820	1.54×10^{-6}
NF-κB	GGGAMTTYCC	0.0757	1.55×10^{-5}
NF-κB (RelA/p65)	GGGRATTTCC	0.0717	8.51×10^{-5}
NF-κB	GGGNNTTTCC	0.0896	1.21×10^{-4}
NF-κB	KGGRAANTCCC	0.0647	6.65×10^{-4}
NF-κB	GGGGAMTTTCC	0.0591	1.66×10^{-3}
IRF1	SAAAAGYGAAACC	0.0560	3.79×10^{-3}
NF-κB	GGGACTTTCCA	0.0532	5.90×10^{-3}
SOX9	AACAATRG	0.0549	6.15×10^{-3}
AP1	TGANTCA	0.0366	7.17×10^{-3}

The top 10 overrepresented TFBS are listed in the order of significance. k/K: extent of overlap with all genes known to possess this motif. Nucleotide nomenclature: K: G or T; M: A or C; N: A, C, G, or T; R: A or G; S: C or G; Y: C or T.
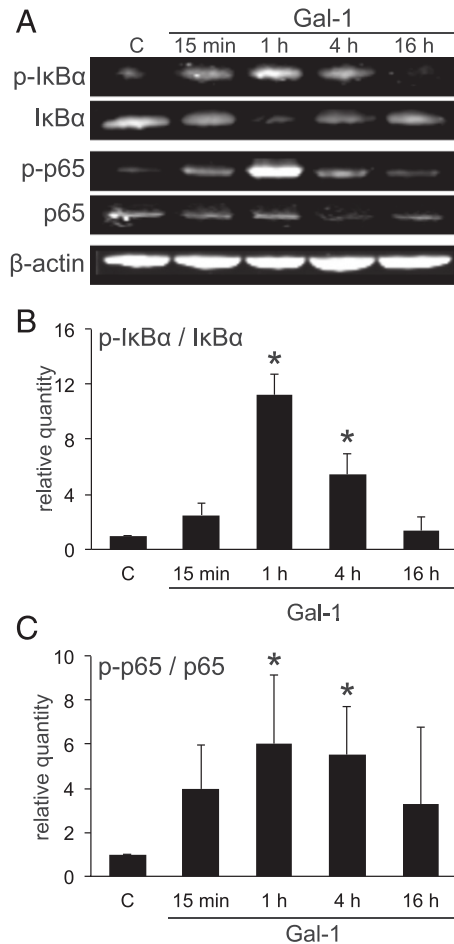
FIGURE 6. Galectin-1 activates the NF-κB pathway in osteoarthritic chondrocytes. (**A–C**) Quantitative Western blot analyses of the phosphorylation of IκBα and p65 in osteoarthritic chondrocytes (n = 3 patients), which were starved overnight and treated with 50 μg/ml Galectin-1 for 15 min, 1 h, 4 h, and 16 h. β-Actin was used as loading control. (A) Shown are the blots of one representative patient for phospho-IκBα (p-IκBα), IκBα, phospho-p65 (p-p65), p65, and β-actin. (B) Shown are the ratios between p-IκBα and IκBα (normalized for β-actin) over time. Data are expressed as relative quantity in comparison with the untreated control set to 1. *$p < 0.05$ (paired t test versus untreated control). (C) Shown are the ratios between p-p65 and p65 (normalized for β-actin) over time. Data are expressed as relative quantity in comparison with the untreated control set to 1. *$p < 0.05$ (paired t test versus untreated control).

1. Fittingly, this treatment led to the phosphorylation of p65 in a time-dependent manner with a peak at 1 h (Fig. 6A, *third lane*), whereas p65 levels were mildly reduced at 4 h (*fourth lane*). Correspondingly, p-p65/p65 ratios were significantly and time dependently increased at 1 and 4 h (Fig. 6C; n = 3 patients; $p < 0.05$), indicating the activation of p65, which sets the stage for nuclear translocation and NF-κB–mediated gene activation.

We next evaluated whether the canonical NF-κB pathway (depicted in Fig. 7A) was of functional relevance to the activity of Galectin-1 in osteoarthritic chondrocytes. We thus specifically blocked several components of the NF-κB pathway using dedicated inhibitors (targets indicated in Fig. 7A) and determined the effect of Galectin-1 on the transcription of *IL1B* as a representative example of an NF-κB–dependent gene.

Fig. 7B–M shows the percentage of Galectin-1 activity in the presence of the inhibitors, with Galectin-1 activity in the absence of inhibitors set to 100%. Dose-dependent and marked impairment of Galectin-1–mediated *IL1B* transcription was observed following specific inhibition of the following: 1) PDK-1 and TAK-1 (Fig. 7B,

7C); 2) the IKK complex components IKKα, IKKβ, and NEMO (Fig. 7E, 7F); 3) IκBα phosphorylation (Fig. 7G) and proteasomal degeneration of IκBα (Fig. 7H); 4) translocation of NF-κB to the nucleus (Fig. 7J, 7K); and 5) the histone acetyltransferases (CBP and p300) and histone deacetylases (Fig. 7L, 7M). In comparison, inhibition of IRAK and TPL-2 resulted in a modest, albeit significant, reduction of Galectin-1 activity (Fig. 7D, 7I, respectively). Evidently, the activation of IRAK and the TPL-2/ERK pathway via NF-κB components might not represent major routes of Galectin-1 signaling in osteoarthritic chondrocytes. Of note, activation for *IL1B* transcription by Galectin-1 was reduced to basal levels in the presence of the cognate sugar lactose in a dose-dependent manner (data not shown).

Taken together, these experiments reveal that blocking of the NF-κB pathway using several different strategies significantly diminished Galectin-1–induced *IL1B* expression. Therefore, canonical NF-κB signaling is likely to play a key role in the gene-regulatory activity of Galectin-1 in osteoarthritic chondrocytes, and this signaling is mediated by glycan-dependent Galectin-1 binding to the cell surface.

Discussion

Until the 1990s, osteoarthritis was thought of mostly as a mechanical, "wear and tear" condition that ultimately leads to cartilage degradation. Several studies have thereafter supported a shift to the concept that inflammation is central to disease progression in both early and late osteoarthritis (38). As to degradation, members of the MMP family, active in breakdown of extracellular matrix in both physiological and pathological processes (39), along with proinflammatory genes are induced via the NF-κB pathway in osteoarthritic chondrocytes. It is now accepted that the combined activities of these pathways are crucial to cause cartilage damage in osteoarthritis (40). The upstream processes are, however, not yet fully identified, and molecular effectors initiating the deleterious signaling cascade are actively sought, as they may offer both etiological insights and novel therapeutic opportunities. Molecular targets that would allow interference with disease onset or progression may indeed have beneficial impact globally on the lives of hundreds of millions of affected individuals.

In previous studies, we had revealed that several members of the galectin family are expressed in vivo in osteoarthritis (10), and that specific features of the chondrocyte glycophenotype are altered during osteoarthritic conditions (8, 9). In this study, we focused on Galectin-1, by immunohistochemical analysis and work with human osteoarthritic chondrocytes. When comparing regions of different degrees of tissue damage, Galectin-1 expression in articular chondrocytes is significantly correlated with the level of cartilage degeneration ($p < 0.0001$). In particular, Galectin-1 accumulated in cartilage zones most closely associated with matrix depletion, chondrocyte clustering, and surface erosion, whereas it was absent in chondrocytes of the deep zone. The pattern of Galectin-1 expression distribution in osteoarthritic cartilage was also congruent with the previously reported staining profiles of MMP1, MMP3, MMP8, and MMP13 as well as IL-1β and TNF-α (41). Taken together, these results suggest a functional link between Galectin-1 and the disease manifestation, possibly through upregulation of proinflammatory cytokines and/or the MMPs. In this concept, Galectin-1 would act as master regulator on a set of disease markers in an auto- and paracrine manner via glycan binding.

To explore whether Galectin-1 has this capacity, we first ascertained the following: 1) Galectin-1 secretion from osteoarthritic chondrocytes using ELISA as well as 2) glycan-dependent binding of Galectin-1 to the chondrocyte surface by Galectin-1-FITC. Testing chondrocytes from clinical specimens, we next addressed the question whether exposure to Galectin-1 could
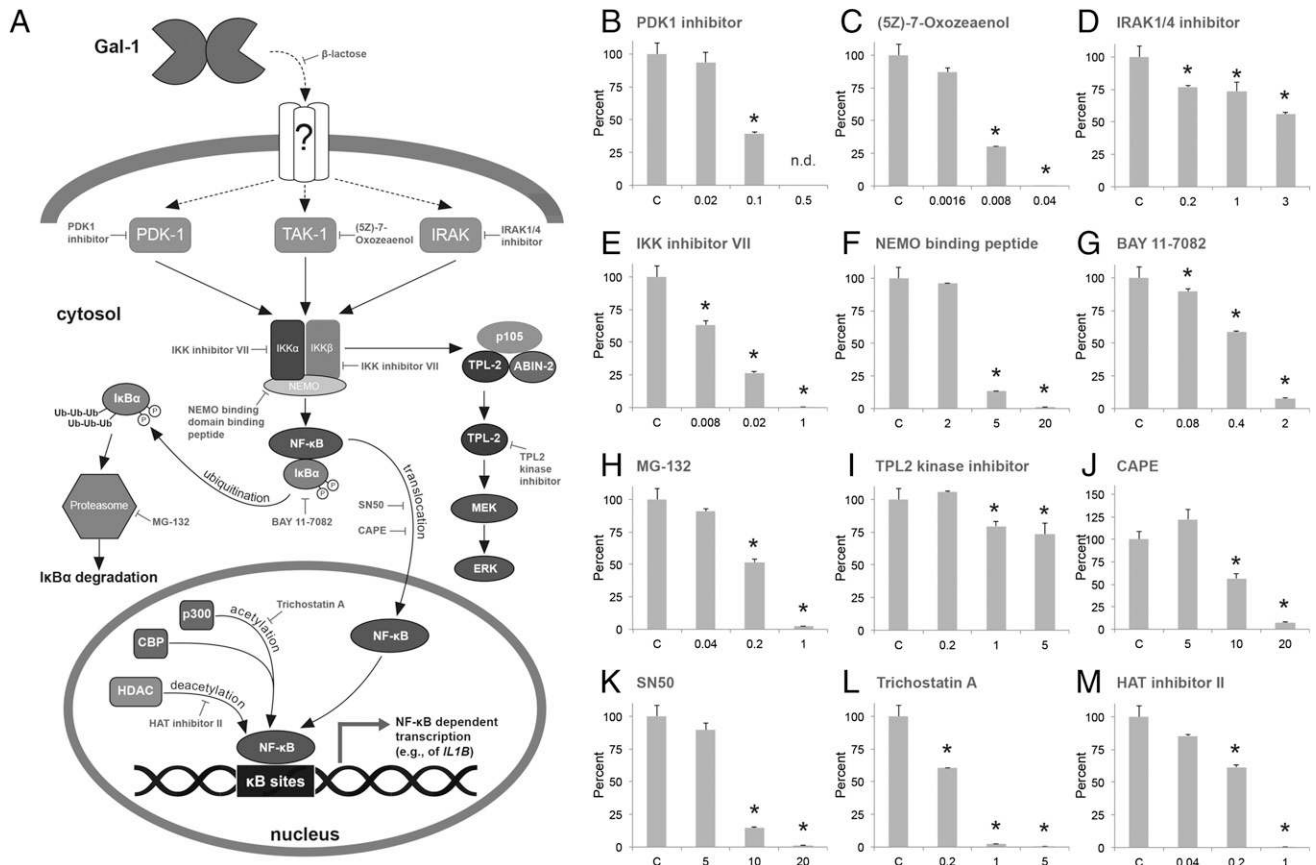
FIGURE 7. Targeted inhibition of NF-κB mediators diminishes Galectin-1–mediated transcription of *IL1B*. (**A**) Scheme of the canonical NF-κB activation pathway. Upon binding of Galectin-1 to osteoarthritic chondrocytes, the NF-κB signaling cascade is induced, leading to transcription of NF-κB–related genes such as *IL1B*. Multiple components and processes of this pathway were blocked by specific inhibitors, as indicated. Dashed arrows indicate currently unknown mechanisms of signal transduction. (**B–M**) Osteoarthritic chondrocytes were starved overnight and treated with 50 μg/ml Galectin-1 in the presence or absence of inhibitors for 24 h prior to RT-qPCR analyses of *IL1B* mRNA levels. The graphs show the percentage of Galectin-1 activity in the presence of the inhibitors (Galectin-1 activity in the absence of inhibitors equals 100%). Labels on x-axes indicate the used concentrations of the inhibitors (μM), which were selected to be nontoxic and to span across the 50% inhibition benchmark whenever possible. The experiment was repeated three times with cells from three different patients. Shown are the mean ± SD (two technical replicates) from one representative experiment. Statistics were performed in comparison with Galectin-1 activity in the absence of inhibitors (100 ± 8.5%), *$p < 0.05$ (unpaired t test).

contribute to known functional characteristics of cartilage degeneration. Biochemical and molecular analyses lend credence to this concept, as Galectin-1 markedly upregulated gene expression of matrix-degrading enzymes (e.g., *ADAMTS4*, *MMP1*, *MMP3*, *MMP13*), and significantly downmodulated extracellular matrix components such as the *AGC1* aggrecan and *COL2A1* collagen. Relevant for cartilage breakdown, ELISA measurements showed that overexpression of the MMP genes is associated with significantly increased secretion of proMMP-1 (6.8-fold), MMP-3 (9.1-fold), and proMMP-13 (113.8-fold). These results, on the levels of production of mRNA and protein, pinpoint Galectin-1 as a potent inducer of extracellular presence of MMPs in osteoarthritic chondrocytes. Similar regulatory events had been reported for Galectin-3 and MMP-1 and MMP-9 in murine B16F10 melanoma cells and for MMP-9 in corneal keratinocytes (42–44), for MMP-7 upregulation by Galectin-7 in murine and human lymphoma cells, and for coexpression of Galectin-7 and MMP-9 in sections of human laryngeal cancer (45, 46). In this context, our finding of enhanced bioavailability of effectors of degeneration prompted us to perform transcriptomic analyses.

Using genome-wide analyses, Galectin-1 was revealed to consistently and strongly trigger an inflammatory expression profile in patients' chondrocytes. This signature highly overlaps with expression profiles in inflammation. Various types of cells and tissues react this way when stimulated with TNF-α, IL-1, LPS, and IKK inhibitors (47, 48), pointing to Galectin-1 as a bona fide proinflammatory coordinator in human chondrocytes. Moreover, the fact that the top six TFBS most significantly associated with genes induced by Galectin-1 are binding sites for NF-κB further intimates the notion that NF-κB is a mediator of the Galectin-1–triggered proinflammatory activities.

Importantly, the lectin itself is not under the control of early proinflammatory signals in chondrocytes, as shown in this work. As the reactivity of mononuclear cells to TNF-α attests (37), extrapolations from experience with a certain cell type are not valid. Because the expression of Galectin-1 can be triggered by various regulators with a strictly cell-type special activity (Supplemental Table II) and can be under the control of a wide variety of transcription factors (data not shown), the search for the pathophysiological elicitor of Galectin-1 overexpression in osteoarthritic cartilage is warranted.

To directly substantiate the involvement of NF-κB downstream of Galectin-1, we showed that Galectin-1 induced the activation of IκBα and p65. Underscoring the salient message of the fundamental difference of Galectin-1 activity in diverse cell types, Galectin-1 impaired proteasomal IκBα degradation in mononuclear cells to attenuate the immune response (37) and reduced the phosphorylation of p65/IKKα/β in human LS-180 colorectal ad-

enocarcinoma cells (49). Moreover, several NF-κB pathway inhibitors impaired Galectin-1–mediated induction of *IL1B* gene expression. These findings are consistent with reports implicating Galectin-1 in the positive regulation of NF-κB gene targets, in contrast to the well-documented anti-inflammatory role. In fact, in human mesenchymal stem cells, Galectin-1 activates NF-κB–dependent fibronectin, laminin-5, and MMP-2 expression (50); in stimulated rat pancreatic stellate cells, Galectin-1 increased the production of chemokines (51, 52); and in tissue of squamous cell, head and neck carcinomas factors associated with NF-κB activation and for poor prognoses such as TRIM23 and MAP3K2 (53).

The genome-wide expression profiling further revealed a number of regulated genes that add to the range of Galectin-1–dependent activities in osteoarthritic chondrocytes. First, we found that *GREM1* and *FRZB* were significantly downregulated by Galectin-1. As both factors had recently been suggested to be natural brakes on terminal hypertrophic differentiation in healthy joint cartilage (54), Galectin-1–dependent regulation of *GREM1* and *FRZB* might contribute to the chondrocyte hypertrophy-like changes reported in osteoarthritic cartilage (55). In addition, Galectin-1 presence modulated expression of genes for components of the extracellular matrix such as integrins, for Galectin-3 and members of other lectin families, including E-selectin, which serves as homing receptor for leukocyte infiltration, as well as the sialyltransferases ST3GAL1 and ST6GAL1, which can reshape the osteoarthritic cartilage glycophenotype and Galectin-1 reactivity.

Having provided these new insights into Galectin-1 functionality in osteoarthritic chondrocytes, a clear direction emerges for further work. In addition to this family member, presence of Galectins-3, -4, and -8 had been revealed in clinical specimens of osteoarthritis (10). As it remains an open question whether they can form a network of regulators, it is an attractive challenge to work with mixtures mimicking the galectin levels in the pathophysiological environment. On the biochemical side, counterreceptor characterization, for the protein/lipid and its glycosylation, will define the first steps of the trigger cascade, from initial binding to signaling. In fact, Galectin-1 is known to target only few glycoconjugates despite the abundance of β-galactosides on the cell surface, the $\alpha_5\beta_1$-integrin being especially relevant for growth regulation (56), and p16^{INK4a}-dependent reprogramming of counterreceptor expression and glycosylation may serve as precedent (27, 28, 57). That the counterreceptor(s), on the level of the protein and/or its glycosylation, may be subject to NF-κB–dependent regulation, as is the case for CD7 (a glycoprotein counterreceptor in apoptosis induction of activated T cells) in murine L7 lymphoma cells (58), is a tempting assumption. These results may disclose clues to identify means to interfere with Galectin-1's chondrocyte-specific disease-promoting effects, for example, by enhancing α2,6-sialylation to preclude Galectin-1 binding or blocking Galectin-1/counterreceptor expression. This strategy based on the reported discovery may lead to an innovative therapeutic approach for osteoarthritis.

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Disclosures

The authors have no financial conflicts of interest.

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