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Enhanced expression of insulin-like growth factor-binding proteins in human osteoarthritic cartilage detected by immunohistochemistry and *in situ* hybridization

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Summary

Objective: To determine the roles of insulin-like growth factor (IGF) and IGF-binding protein (IGFBP) in the pathogenesis of osteoarthritis (OA).

Design: Cartilage tissues were obtained from the femoral heads of patients with OA, and those from patients with femoral neck fractures were used as a control. The expression of IGFBP-3, -4, and -5 was examined using immunohistochemistry and *in situ* hybridization, and IGF-I and IGF-I receptors were also immunohistochemically detected. The percentages of positive chondrocytes were determined by counting the total number of chondrocytes over the area of the surface, middle, and deep zones of the cartilage.

Results: There was a marked increase in the percentage of positive chondrocytes in all IGFBPs on protein and messenger RNA levels for OA compared to that of the control cartilage. Furthermore, enhanced expression of IGFBPs and the IGF-I/IGF-I receptor was positively correlated with the histologic score for cartilage lesions.

Conclusion: Up-regulation of IGFBPs as well as IGF-I and its receptor was observed for OA cartilage tissue, suggesting the involvement of IGFBPs in the pathogenesis of OA.

Key words: Insulin-like growth factor, Osteoarthritis, Cartilage, IGF-binding protein.

Introduction

Osteoarthritis (OA) is characterized by degeneration of the cartilage matrix and gradually progresses without repair of the damaged tissue, leading to functional disability of the joints. No effective therapy for protecting cartilage from degeneration has yet been established. The etiology of the disease has not been fully understood, but aging and mechanical stress are considered to be possible associated factors[1].

Joint destruction is caused by an imbalance between the synthesis and degradation of the cartilage matrix. For the metabolism of cartilage matrix, interleukin (IL)-1β and tumor necrosis factor (TNF-α) act as destructive cytokines[2], while insulin-like growth factor (IGF)-I and members of the transforming growth factor-β family act as growth promoting factors[3,4]. These negative and positive factors of matrix metabolism are produced by chondrocytes and act in a paracrine and/or autocrine fashion[5]. In OA, elevated levels of IGF-I occur in the synovial fluids[6,7], along with the increased expression of IGF-I in OA chondrocytes[8,9]. Moreover, expression of the IGF-I receptor in OA cartilage is normal or increased[10,11]. These findings led us to assume the involvement of IGF-I in the pathogenesis of OA. However, the hyporesponsiveness of chondrocytes to IGF-I was reported for experimental arthritis induced by the injection of zymosan into murine knee joints[12] or for patients with OA[13].

Until now, six IGF-binding proteins (IGFBP-1 to -6), which bind to IGF-I or IGF-II with high affinity[14], have been discovered in the serum. In addition, IGFBP-2 to -5 are synthesized and secreted by human articular chondrocytes *in vitro*[15]. In the synovial fluid of OA patients, elevated levels of IGFBP-3 and -4 expression can be observed by Western ligand blotting methods[16], and the increased expression of IGFBP-3 and -5 messenger RNA (mRNA) in OA chondrocytes can be seen by Reverse Transcription (RT)-quantitative polymerase chain reaction (PCR)[15]. Since IGF-I has a greater affinity for IGFBPs than its receptor, these results suggest that the enhanced production of IGFBP-3, -4, or -5 may be involved in the pathogenesis of OA by decreasing the effective concentration of IGF-I. However, little is known about the cellular expression pattern of IGFBPs in OA cartilage.

In this study, we examined the expression of IGFBP-3, -4 and -5 in decalcified tissue sections of OA and normal cartilage at the transcript and protein level using *in situ* hybridization (ISH) and immunohistochemistry (IHC), respectively. Additionally, IGF-I and IGF-I receptors were immunohistochemically detected. We found that the expression of IGFBP-3 to -5, as well as IGF-I and its receptor was significantly increased in OA cartilage, compared to that of normal aged cartilage, suggesting involvement of the of IGF-axis in the pathogenesis of OA.

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Materials and methods

CARTILAGE SPECIMENS

Human articular OA cartilage specimens were obtained from the femoral heads of 10 female patients (mean age of 55, range of 43–72) while undergoing total hip replacement with informed consent. The diagnosis of OA was based on clinical and radiologic evaluations[17]. As a control, aged cartilage tissue was obtained from the femoral heads of 10 female patients (mean age of 76, range of 51–85) with a femoral neck fracture while receiving a hip prosthesis. All specimens were immunohistologically examined, while six from the OA group and five from the control group were examined using ISH.

TISSUE PREPARATION

Full thickness pieces of cartilage were obtained from the weight bearing area of the joint immediately after surgery. The samples were then fixed in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) pH 7.4 at 4°C for 12 h, decalcified with 10% ethylenediaminetetraacetic acid (EDTA) at 4°C for 5–7 days and embedded in paraffin as previously detailed[18]. The tissue blocks were next cut into five-micron-thick sections, mounted on aminopropyltriethoxysilane-coated glass slides (Matsunami, Tokyo, Japan) and used for the following experiments.

HISTOLOGIC GRADING

The sections were stained with hematoxylin and eosin, Safranin-O and Fast Green for histological examination. The severity of the OA cartilage lesions was determined using the histologic/histochemical scores described by Mankin *et al.*[19]; loss of Safranin-O staining (0–4), cellular changes (0–3), invasion of a tidemark by blood vessels (0–1), and structural changes (0–6), where 0 indicates the state of normal cartilage and 14 indicates the most severe histological change (Table I).

Table I
Histological–histochemical grading system (Mankin et al.[19])

	Grade
I Structure	
(a) Normal	0
(b) Surface irregularities	1
(c) Pannus and surface irregularities	2
(d) Clefts to transitional zone	3
(e) Clefts to radial zone	4
(f) Clefts to calcified zone	5
(g) Complete disorganization	6
II Cells	
(a) Normal	0
(b) Diffuse hypercellularity	1
(c) Cloning	2
(d) Hypocellularity	3
III Safranin-O staining	
(a) Normal	0
(b) Slight reduction	1
(c) Moderate reduction	2
(d) Severe reduction	3
(e) No dye noted	4
IV Tidemark integrity	
(a) Intact	0
(b) Crossed by blood vessels	1

IMMUNOHISTOCHEMICAL STUDIES

The paraffin-embedded sections were deparaffinized and then rehydrated. After the inactivation of endogenous peroxidase with 0.3% H_2O_2 in methanol at room temperature (RT) for 30 min, the slides were preincubated with 1% bovine serum albumin (BSA) in PBS for 1 h to block nonspecific reactions. The sections were then incubated with the primary antibody (rabbit polyclonal anti-human IGFBP-3, -4, or -5 serum, mouse monoclonal anti-human IGF-I IgG or chicken polyclonal anti-human IGF-I receptor IgY) (UBI, Lake Placid, NY) overnight at RT. After washing four times with 0.075% Brij in PBS, the slides were incubated with second antibody [horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG, HRP-conjugated goat anti-mouse IgG or HRP-conjugated rabbit anti-chicken IgY] for 1 h at RT. After washing next with 0.075% Brij in PBS three times, the HRP sites were visualized with 3,3′-diaminobenzidinetetrahydrochloride (DAB) and H_2O_2, and counterstained with 2% methylgreen as previously detailed[20]. As a negative control, rabbit nonimmune serum, normal mouse IgG or normal chicken IgY was used instead of the primary antibody. The percentages of positive chondrocytes were determined by counting the total number of chondrocytes over the area of the surface, middle, and deep zones of the cartilage, which contained between 70 and 500 cells with a mean of 152. The sections were subsequently examined under a light microscope (Olympus Provis AX 80) at a magnification of 400× where five fields in the section were selected at random.

OLIGO-DNAs

The antisense oligo-DNA sequences which were complementary to part of the sense sequences for human IGFBP-3[21], -4[22] and -5[23] were selected as shown in Table II. We also prepared oligo-DNA with 34 bases which was complementary to part of the human 28S ribosomal RNA

Table II
The T–T dimerized oligo-DNA probe sequences of human IGFBPs for in situ hybridization

Antisense oligo-DNA probe
IGFBP-3
5′: TTATTA-CGG TGT AGA TGC CGC ACG GCT GGC CCT CGC TCA GTG CGC ACG TCA-ATTATT: 3′
IGFBP-4
5′: TTATTA-TCC TGG ATG GCC TCG ATC TCC GCC AGC TCC ATG CAC ACG CCT-ATTATTATT: 3′
IGFBP-5
5′: TTATTA-CCC TGC TCA GAC TCC TGT CTC ATC TCA GGT GCA GAG ATG ATC CGATTATTATT: 3′
Sense oligo-DNA probe
IGFBP-3
5′: TTATTA-TGA CGT GCG CAC TGA GCG AGG GCC AGC CGT GCG GCA TCT ACA CCG-ATTATTATT: 3′
IGFBP-4
5′: TTATTA-AGG CGT GTG CAT GGA GCT GGC GGA GAT CGA GGC CAT CCA GGA-ATTATTATT: 3′
IGFBP-5
5′: TTATTA-CGG ATC ATC TCT GCA CCT GAG ATG AGA CAG GAG TCT GAG CAG GG-ATTATTATT: 3′

IGFBP; insulin-like growth factor binding protein; T-T; thymine-thymine.

(rRNA) as a positive control probe[24], and this was used to assess the integrity and hybridizability of RNA in the cartilage tissue sections. A computer-assisted search (GenBank nucleic acid sequence database Release 110.0) of the selected oligo-DNA sequences (without the ATT repeats) was conducted and no significant homology with any other published sequences was found.

LABELING OF OLIGO-DNAS

The oligo-DNAs were haptenized by ultraviolet-irradiation to form thymine–thymine (T–T) dimers as previously detailed[25].

DOT-BLOT HYBRIDIZATION

The procedures for dot-blot hybridization were previously described[24,26]. Briefly, 2 μl of the sense oligo-DNA solutions were placed on nitrocellulose membranes that had been pretreated with 20× SSC (1× SSC = 0.15 M sodium chloride and 0.015 M sodium citrate, pH 7.0) in a series of spots from 1 pg to 10 ng per spot. The membranes were hybridized at 42°C for 15–17 h with a 2 μg/ml T–T dimerized corresponding antisense or sense probe. After washing once with 2× SSC, once with 1× SSC, and once with 0.5× SSC, the membranes were immersed in blocking solution [5% BSA, 100 μg/ml yeast transfer RNA (tRNA), 100 μg/ml salmon sperm DNA, 0.6 M NaCl, PBS and 500 μg/ml normal mouse IgG] for 1 h. Reaction with HRP linked mouse anti-T–T IgG (Kyowa Medex, Japan, diluted at 1:80 with the blocking solution) was performed for 3 h and after washing with PBS the HRP sites were visualized with DAB, H_2O_2, Co^{2+}, and Ni^{2+} according to Adams[27].

When T–T dimerized IGFBP antisense probes were hybridized with IGFBP sense oligo-DNAs fixed on a nitrocellulose filter, 10 pg of each oligo-DNA was detected. However, no staining was found with the sense probe. Similar results were obtained for the other probes, indicating that the antisense probes were specific and possessed adequate sensitivity for ISH studies.

IN SITU HYBRIDIZATION

ISH was performed as previously described[26,28]. Briefly, the sections were deparaffinized and rehydrated, and then treated with 0.2 N HCl at RT for 20 min and digested with 10 μg/ml of proteinase K at 37°C for 15 min. After

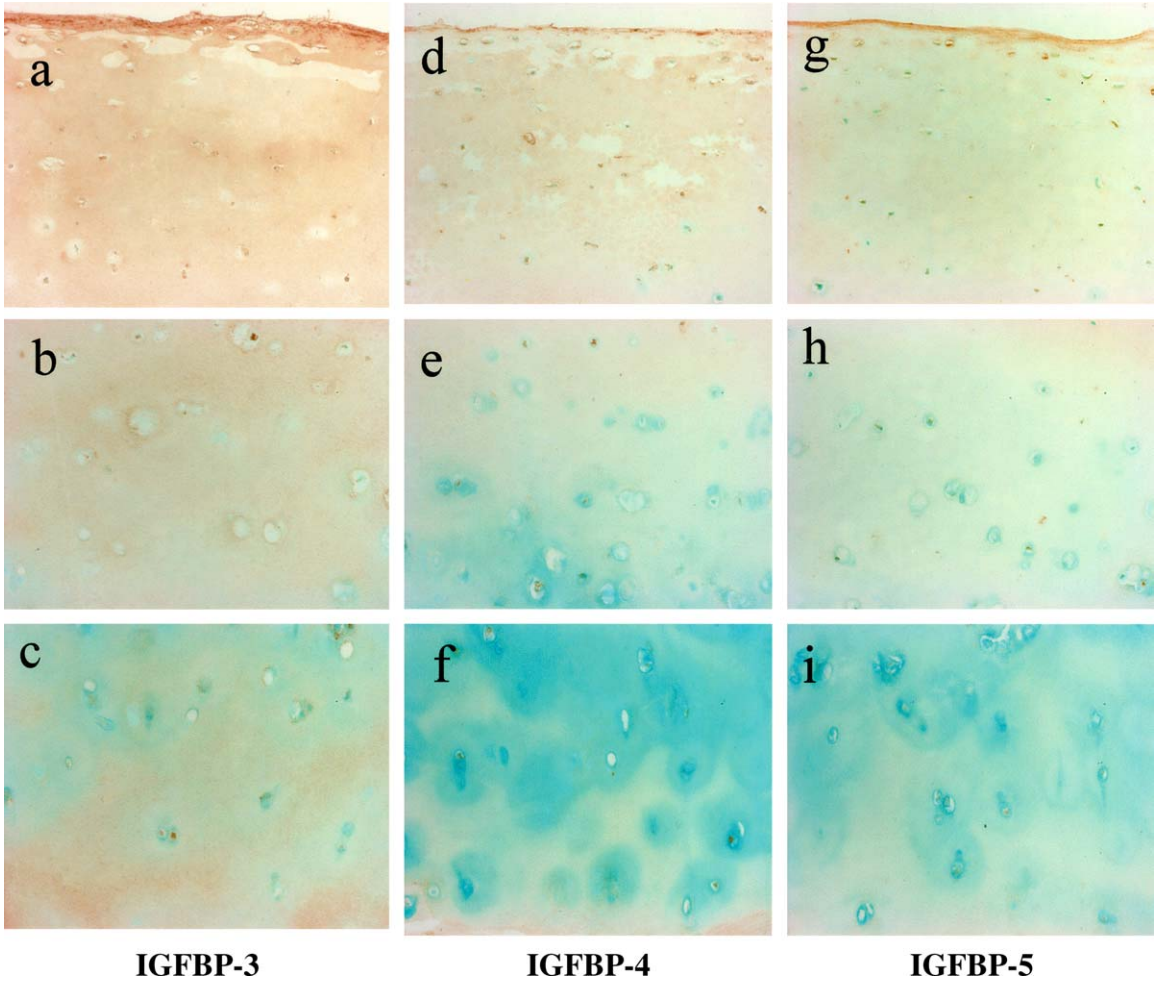

Fig. 1. IHC for the detection of IGFBP-3 (a, b, c), -4 (d, e, f), and -5 (g, h, i) for representative sections of control articular cartilage from a 74-year-old female. The zonal distribution of IGFBP immunostained chondrocytes is shown: superficial layer (a, d, g), middle layer (b, e, h), and deep layer (c, f, i). (Original magnification 200×.)

post-fixation with 4% PFA in PBS for 5 min, they were immersed twice in 2 mg/ml glycine in PBS for 15 min and kept in 40% deionized formamide in 4× SSC until being used for hybridization. Hybridization was carried out at 37°C overnight with a 1–4 μg/ml T–T dimerized oligo-DNA probe dissolved in hybridization medium composed of 10 mM Tris–HCl (pH 7.4), 1 mM EDTA, 0.6 M NaCl, 1× Denhardt's solution, 250 μg/ml yeast tRNA, 125 μg/ml salmon sperm DNA, and 40% deionized formamide. After washing twice with 50% formamide in 0.5× SSC followed by 50% formamide in 2× SSC three times at 37°C for 1 h each time, the signals were detected using enzyme-IHC as described above, without any counterstaining.

QUANTIFICATION OF ISH

We quantitatively analyzed the signal intensity of ISH with an image analyzer (AXIOVISION Application version 2.0.5.3, Carl Zeiss Vision GmbH) connected to a Carl Zeiss CCD camera (AxioCam). The images were transformed into a matrix of 650 × 514 pixels per frame with each pixel having one of 256 gray levels ranging from 0 corresponding to black to 255 corresponding to absolute white. The frequencies of positive cells for each IGFBP mRNA, which were determined as the number of cells stained with the antisense probe divided by the levels of staining with the sense control probe, were measured using the public domain Scion Image analysis program (Scion Corporation, Maryland, USA). The number of positive chondrocytes was counted by examining at least 100 chondrocytes for each surface, middle and deep zone of the cartilage, and the frequency was expressed as a percentage.

STATISTICAL ANALYSIS

The data were expressed as means ± S.E.M. and analyzed using the Mann–Whitney *U* test by Statview-J4.5 computer software (Abacus Concepts Inc., Berkeley, USA). A *P*-value of less than 0.05 was considered to indicate statistical significance. Correlations between the different parameters were calculated using Spearman's rank correlation coefficient.

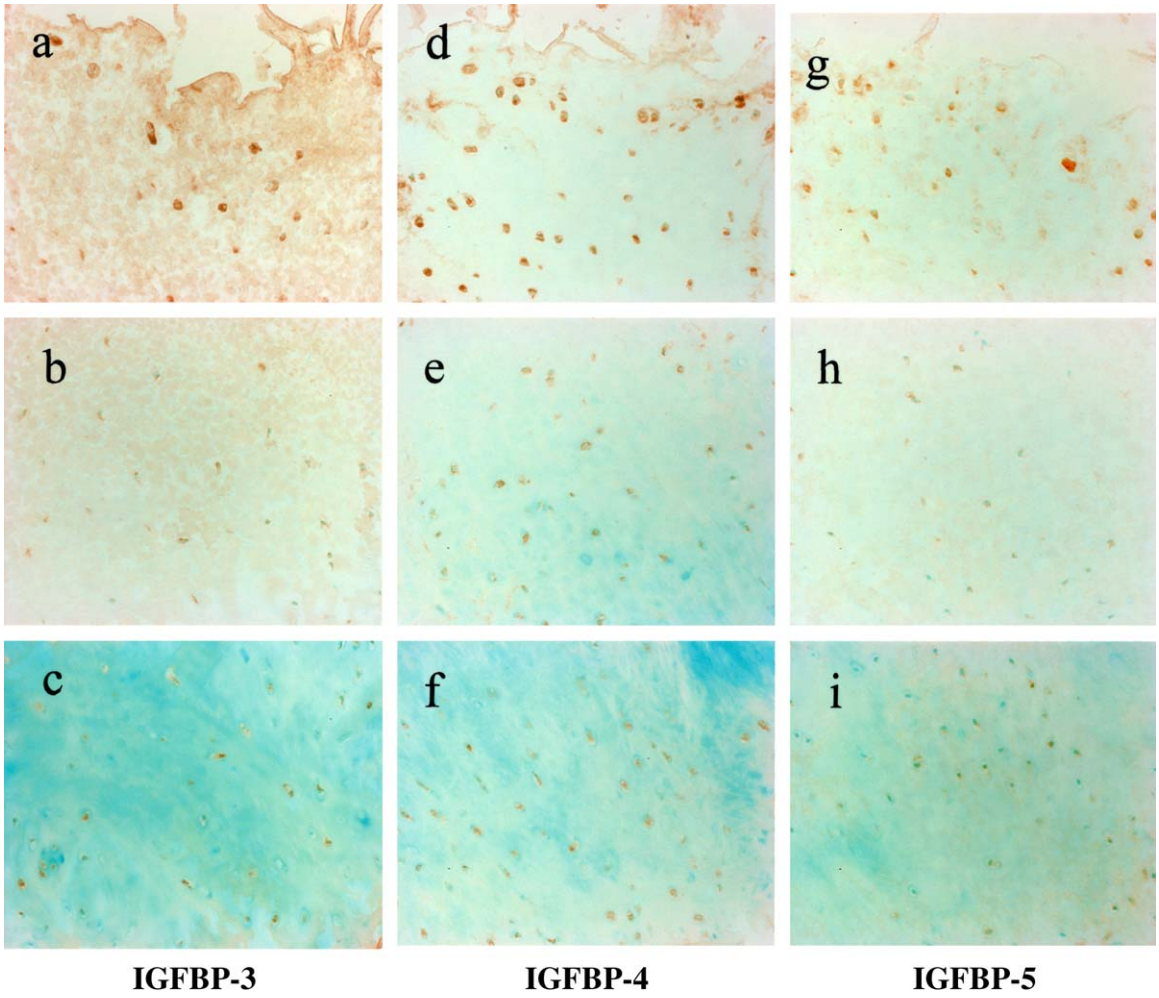

Fig. 2. IHC for the detection of IGFBP-3 (a, b, c), -4 (d, e, f), and -5 (g, h, i) for representative sections of osteoarthritic cartilage from a 62-year-old female. The zonal distribution of IGFBP immunostained chondrocytes is shown: superficial layer (a, d, g), middle layer (b, e, h), and deep layer(c, f, i). (Original magnification 200×.)

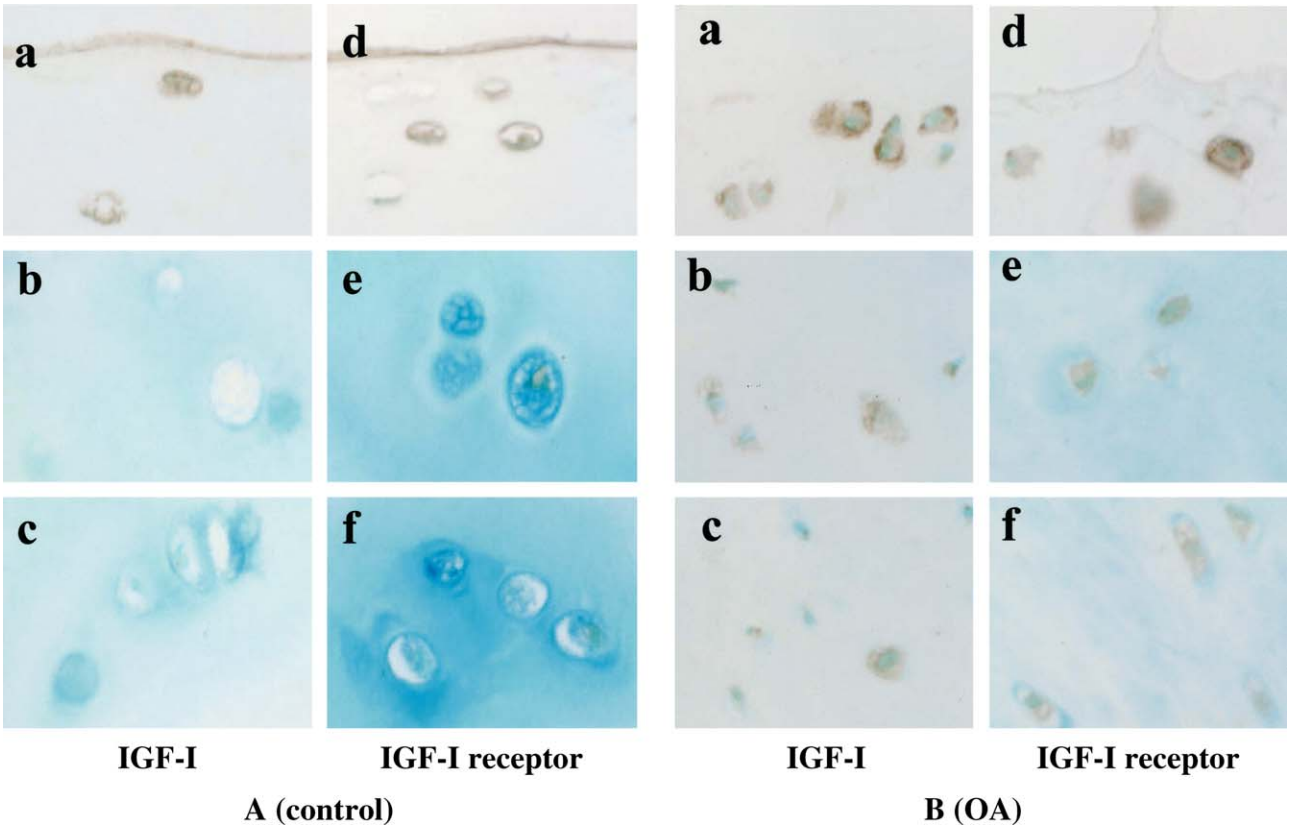

Fig. 3. IHC of IGF-I and the IGF-I receptor in representative sections of control articular cartilage from a 74-year-old female (A) and osteoarthritic cartilage from a 62-year-old female (B). The zonal distribution of immunostained chondrocytes is shown: superficial layer (a, d), middle layer (b, e), and deep layer (c, f). (Original magnification 400×.)

Results

HISTOLOGIC FINDINGS

The OA cartilage tissues were categorized with Mankin scores ranging from 6 to 12 in the (average 8.6) based on the degree of fibrillation, clustering of cells, and loss of Safranin-O staining. There were essentially no histologic abnormalities in the control cartilage samples except for a decrease in Safranin-O staining in some cases. The Mankin score for the control specimens ranged from 0 to 2 with an average of 0.7.

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Representative immunohistochemical staining for IGFBP-3, -4, and -5 in the control and OA cartilage is shown in

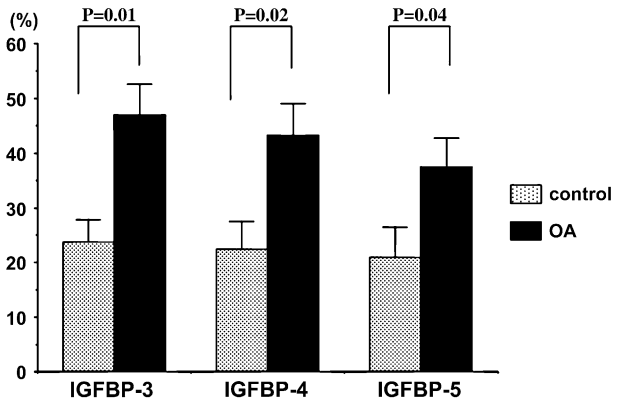

Fig. 4. A histogram of the percentage of cells immunoreactive against IGFBP-3, -4, and -5 in the control ($n = 10$) and OA ($n = 10$) cartilage over all layers. The bars indicate the mean and S.E.M. score and the *P*-values indicate the difference between the control and OA cartilage calculated using the Mann–Whitney *U* test.

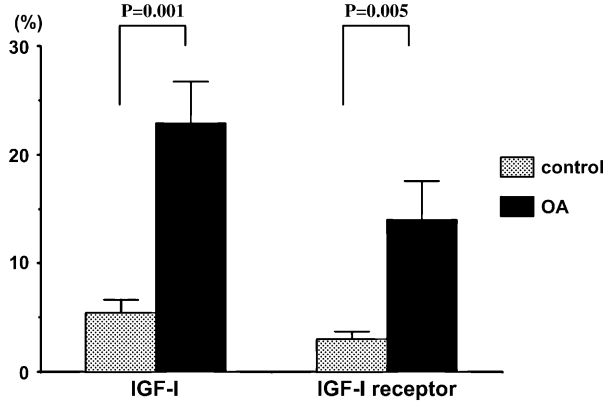

Fig. 5. A histogram of the percentage of cells immunoreactive against IGF-I and the IGF-I receptor in the control ($n = 10$) and OA ($n = 10$) cartilage over all layers. The bars indicate the mean and S.E.M. score and the *P*-values indicate the difference between the control and OA cartilage calculated using the Mann–Whitney *U* test.

Figs. 1 and 2, for which the expression of IGFBP-3, -4, and -5 in each layer of the cartilage, surface, middle and deep zones was evaluated. IGFBP-3, -4 and -5 were found in the cytoplasm of chondrocytes in all layers in the normal and OA cartilage, while more intense signals and an increased number of positive chondrocytes for these three IGFBPs was found in the OA cartilage. In addition, staining of the cartilage matrix for IGFBP-3 seemed to be increased, while that for IGFBP-4 or -5 was nearly constant. When adjacent sections were reacted with normal rabbit serum instead of primary antibody, no staining was observed (data not shown).

Representative immunohistolocalization of IGF-I and the IGF-I receptor in the cartilage is shown in Fig. 3. For the control cartilage, chondrocytes stained for IGF-I or the IGF-I receptor were especially numerous in the surface layer, whereas staining was seldom observed in the middle and deep layers [Fig. 3(A)]. For the OA cartilage, an increased number cells stained for IGF-I or the IGF-I receptor was noted for the surface layer, and some chondrocytes were also stained in the middle and deep layers [Fig. 3(B)].

The percentages of positive chondrocytes for IGFBP-3, -4, and -5 were evaluated for all layers of the samples for the control ($n = 10$) and OA cartilage ($n = 10$) (Fig. 4). The percentage of cells immunostained for IGFBP-3, -4, and -5 was significantly higher for the OA cartilage compared to the control ($P = 0.01$, $P = 0.02$, $P = 0.04$, respectively). Likewise, the percentages of positive chondrocytes immunostained for IGF-I or the IGF-I receptor were evaluated for all layers for samples from the control ($n = 10$) and OA cartilage ($n = 10$) (Fig. 5). The percentage of cells immunostained for IGF-I or the IGF-I receptor was significantly higher for the OA cartilage compared to the control ($P = 0.001$, $P = 0.005$, respectively). Additionally, we compared these findings to the histological Mankin scores for cartilage lesions. Positive relationships were found between the Mankin score and the percentage of immunostained cells for IGFBP-3, 4, and -5 ($r = 0.74$,

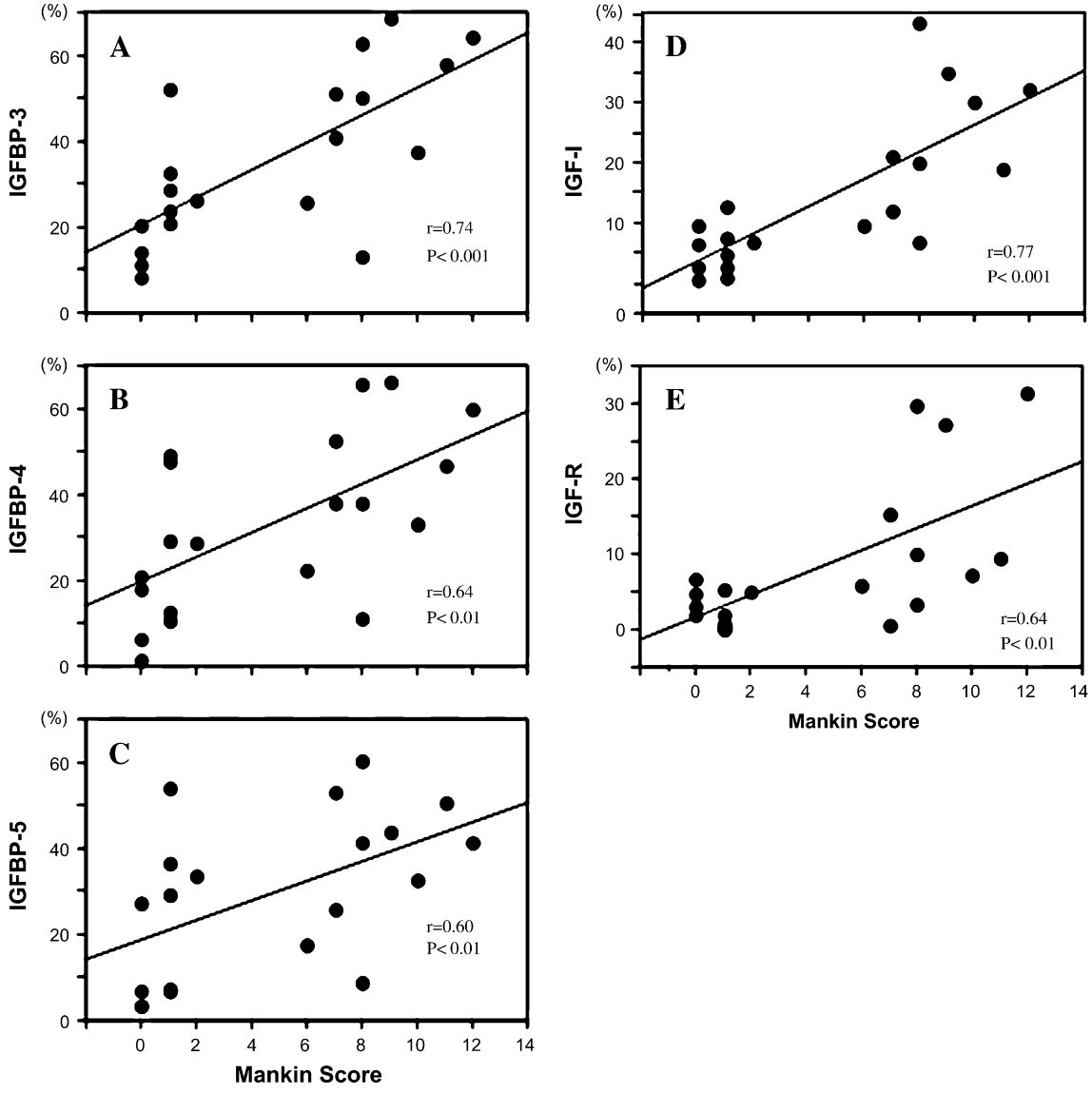

Fig. 6. The relationship between the Mankin score and IGFBP-3 (A), IGFBP-4 (B), IGFBP-5 (C), IGF-I (D), and IGF-I (E). The Spearman's rank correlation coefficient and *P*-value are also shown.

$r = 0.64$, $r = 0.60$, respectively). Also, IGF-I and the IGF-I receptor were found to be correlated with the histological score ($r = 0.77$, $r = 0.64$, respectively) (Fig. 6).

ISH ANALYSIS FOR IGFBP-3, -4, OR -5 MRNA EXPRESSION

Representative results of ISH for the control and the OA cartilage are shown in Figs. 7 and 8. Although the signal intensities for IGFBP-3, -4, or -5 mRNA varied among the specimens, IGFBP-3, -4, and -5 mRNA were expressed in all layers of the cartilage tissue. Compared to the control cartilage, the expression of IGFBP-3, -4 and -5 mRNA was increased in all layers of the chondrocytes for the OA Cartilage. As a positive control, when a section was hybridized with T−T dimerized 28S rRNA, signals were observed in chondrocytes in all zones of the OA and control cartilage (data not shown). As a negative control, when a section was hybridized with T−T dimerized sense probes, no signals were observed (data not shown).

When the percentages of chondrocytes expressing IGFBP-3, -4, and -5 mRNA per total cells were evaluated for the control ($n = 5$, mean age of 77, range of 68 to of 85, Mankin score average of 1) and OA cartilage ($n = 6$, mean age of 51, range of 43 to 62, Mankin score average of 8.5) (Fig. 9), the expression of IGFBP-3, -4, and -5 mRNA was significantly increased in the OA cartilage compared to that of the control specimens ($P < 0.01$, $P < 0.01$, and $P < 0.02$ respectively), and this was consistent with result for the immunohistochemical study.

Discussion

In this study, we investigated the expression of IGFBP-3, -4, and -5 at the protein and mRNA levels in OA cartilage, and found that the expression of these proteins was significantly increased in OA cartilage compared to that of the control. Also increases in the expression of IGF-I and the IGF-I receptor were immunohistochemically confirmed for the OA cartilage. These findings in part suggest that any disorder in the maintenance of cartilage in the OA specimens was not due to a decrease in IGF-I or the IGF-I receptor. Locally produced IGFBPs act as autocrine/paracrine regulators of IGF activity in a positive and negative manner. The inhibitory effect of IGFBPs is mediated via the blocking of IGF binding to the IGF receptor. On the other hand, IGFBPs on the cell surface or in the extracellular matrix increase the local concentration of IGFs, thereby,

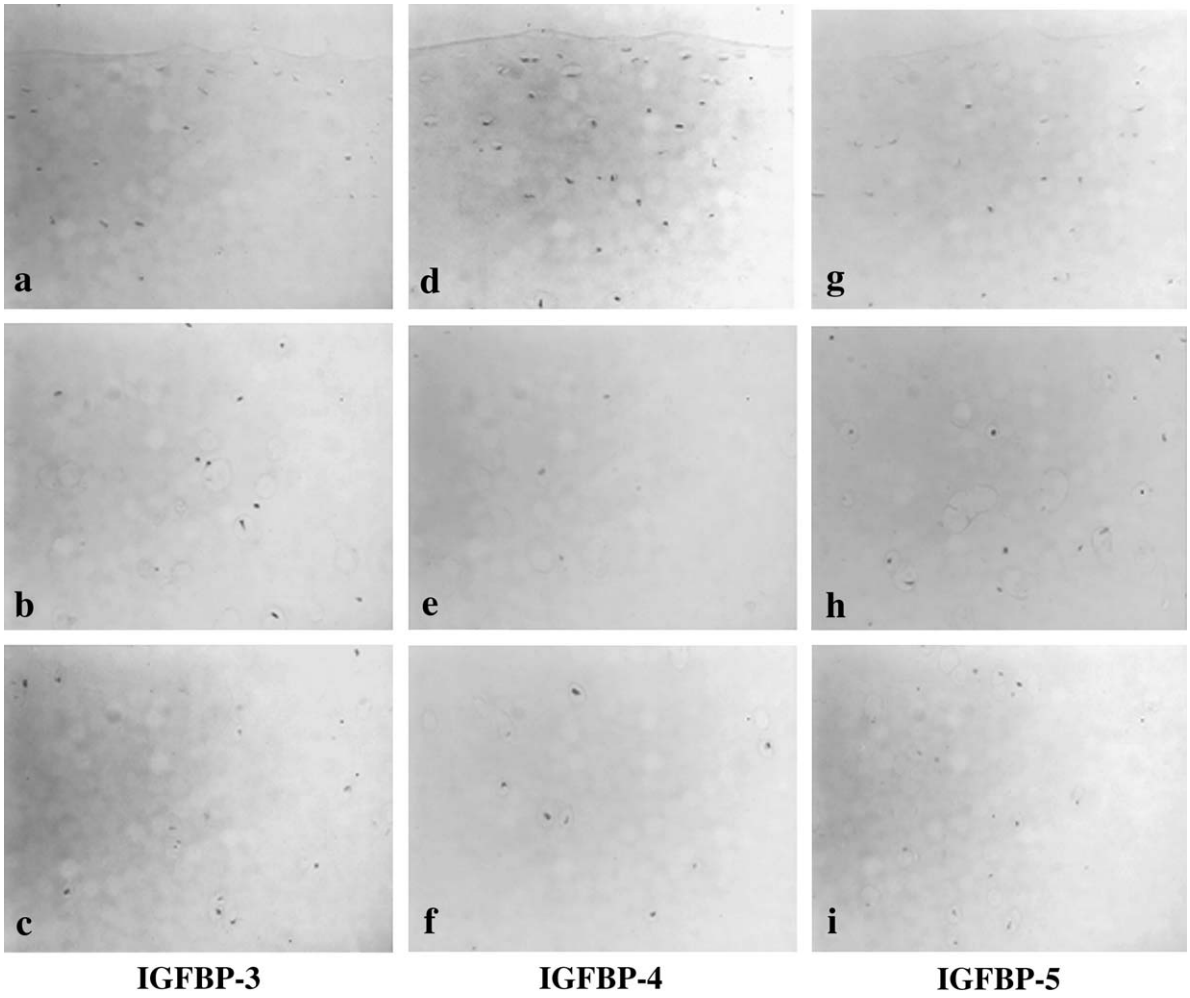

Fig. 7. ISH in representative sections of control cartilage from a 74-year-old female. ISH was carried out using T−T dimerized IGFBP-3 (a, b, c), -4 (d, e, f) or -5 (g, h, i) antisense probes. The zonal distributions of IGFBP mRNA expression of are shown: surface layer (a, d, g), middle layer (b, e, h), and deep layer (c, f, i). (Original magnification 200×.)

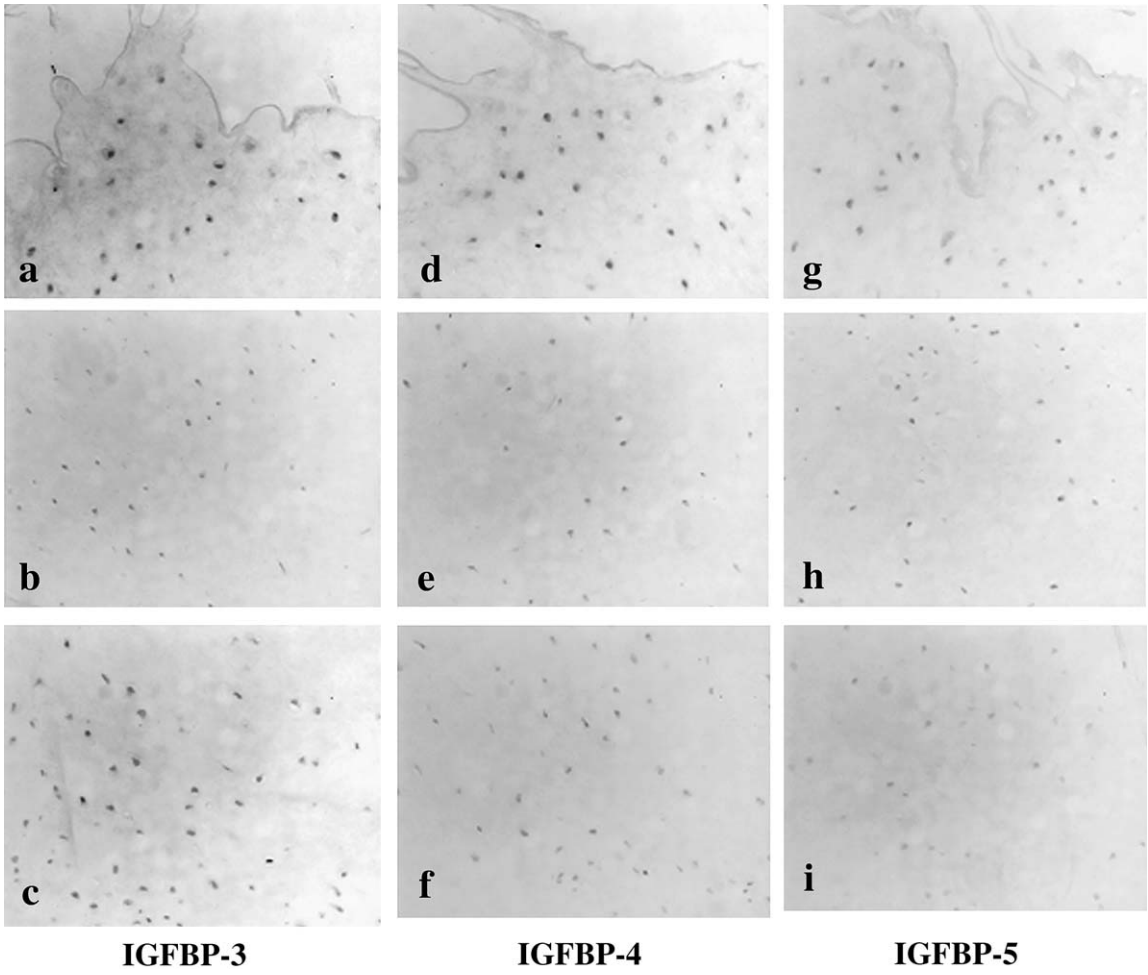

Fig. 8. ISH in representative sections of osteoarthritic cartilage from a 62-year-old female. ISH was carried out using T–T dimerized IGFBP-3 (a, b, c), -4, (d, e, f) or -5 (g, h, i) antisense probes. The zonal distributions of IGFBP mRNA expression of are shown: surface layer (a, d, g), middle layer (b, e, h), and deep layer (c, f, i). (Original magnification 200×.)

potentiating this IGF activity. Furthermore, some IGFBPs also act independently of IGFs[29]. In a previous study, we showed that the binding of IGF-I to cells is displaced by IGFBP-3 and IGFBP-5[30], suggesting that the enhanced expression of IGFBPs in OA cartilage results in the neutralization of IGF-1 activity. However, further studies are needed to clarify these mechanisms.

Although Olney *et al.* showed the existence of IGFBP-2, -3 and -4 but not IGFBP-5 in the conditioned medium of normal human chondrocytes *in vitro*[31], IGFBP-5 was detected at the almost the same level as IGFBP-3 and -4 in our study. To explain this discrepancy, it is possible that human chondrocytes also produce IGFBP-5 *in vitro*, but that IGFBP-5 produced in the medium might be degraded by an IGBFP-5 protease. The presence of an IGFBP-5 protease has been identified in the medium of chondrocytes[32,33]. In contrast, it was reported that the inhibition of IGFBP-5 proteolysis in joint fluid increases IGFBP-5[34], which leads to the improvement of OA.

IGF-I increases the production of IGFBP-3, -4 and -5 by chondrocytes[30,35], and the up-regulation of IGF-I mRNA and IGF-I protein in the surface area of fibrillated OA cartilage has also been reported[9]. These findings suggest that the increased level of IGF-I secreted by chondrocytes

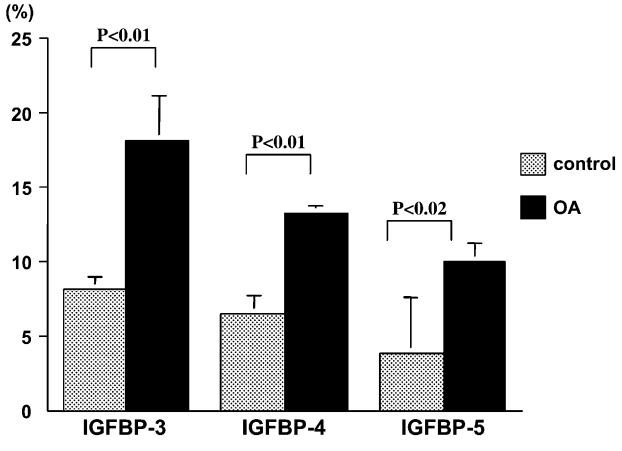

Fig. 9. A histogram of the percentage of chondrocytes expressing IGFBP-3, -4, and -5 mRNA evaluated over all zones for samples from the control ($n = 5$) and OA cartilage ($n = 6$). The bars indicate the mean and S.E.M. score and the *P*-values indicate the difference between the control and OA cartilage calculated using the Mann–Whitney *U* test.

may increase the expression of IGFBPs through an autocrine/paracrine mechanism in the OA tissues.

Various factors known regulate the production of IGFBPs. Increased IGFBP-3 levels in articular cartilage were also immunohistochemically observed for estrogen treated monkeys[36]. Inflammatory cytokines such as IL-1α or TNF-α increase the production of IGFBP-3 or -5 by chondrocytes[31], and prostaglandin E2 also increases the expression of IGFBP-3 and -4[37,38]. These cytokines are elevated in inflammatory diseases such as Rheumatoid Arthritus (RA) and OA, and play roles in the destruction of cartilage tissue in OA. Furthermore, IL-1β and TNF-α have been immunodetected on the surface[39] and in the middle and deep layers[40] of OA cartilage. Therefore, these cytokines may exert degenerative effect on cartilage through the enhanced expression of IGFBPs in OA tissue.

The increased level of IGFBP-3 in OA cartilage was also seen by directly analyzing cartilage tissue extract[41] or by quantifying locally synthesized IGFBP-3 from cartilage explants[42]. However, little is known about the localization of IGFBPs in human articular cartilage, as only IGFBP-3 was identified in the chondrocyte territorial matrix together with fibronectin[43,44]. This complex of IGFBP-3 and fibronectin stores IGF-I in the chondrocyte territorial matrix, which helps maintain a relatively constant level of IGF-I. In this study, we found that expression of the IGF-I receptor was significantly increased in OA cartilage and was correlated with the histological degeneration score. These results are consistent with the findings of Middleton *et al.*[10], showing increased expression of IGF-I receptor mRNA in OA cartilage and the highest level in advanced lesions by means of ISH. On the other hand, similar levels of IGF-I receptor mRNA in normal and OA chondrocytes were reported by Tardif *et al.* using quantitative RT-PCR[11]. These discrepancies might have occurred due to the stage of OA[45] or the age of the patients. Allowing for the fact that the mean age of patients in control group was higher than those in OA group in this study, there is still room for argument of the results.

In conclusion, we found that the expression of IGFBP-3, 4 and -5 was significantly increased in OA cartilage as well as is IGF-I and the IGF-I receptor compared with that of control cartilage. Moreover, the enhanced expression was significantly correlated with the histological changes in the lesions. These results indicate that IGFBPs locally produced by chondrocytes may play a role in the pathogenesis of OA.

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