Articular cartilage was collected from patients with OA or femoral neck fracture for the use of these proteins in combination with conventional OA biomarkers may better reflect the grade and prognosis of OA.

Introduction

Because patients with osteoarthritis (OA) outnumber those with rheumatoid arthritis (RA) or even osteoporosis, there is a need to identify molecular markers and develop new treatments against this disease. The primary pathology of OA is degeneration of the articular cartilage matrix. Recent epidemiological investigations have revealed that acetabular dysplasia is the most important factor involved in OA onset in the hip joint (Jingushi et al., 2011). Obesity has also been identified as closely associated with OA in knee and hip joints. Such mechanical stresses cause abnormalities in the metabolism of articular cartilage, leading to degeneration of the cartilage matrix. Although this degeneration has been attributed to various possible mediators, including proteases, hypoxia inducible factor-2α (Hirata et al., 2012; Saito et al., 2010; Saito & Kawaguchi, 2010), reactive oxygen species (ROS) (Xie et al., 2012), and the complement system (Wang et al., 2011a), the precise molecular pathogenic mechanism of OA remains unknown. Accordingly, current treatment practices are limited to surgical procedures incorporating prosthetic joint replacement with medical therapies limited to symptom control (Yamada et al., 2009). Curative non-surgical treatments remain to be established. OA is associated with a particularly high morbidity rate among the elderly. Because surgical procedures can be major physical challenges for the elderly owing to complications from cardiovascular disease and diabetes, the demand for the development of medical therapies for OA is also crucial from the perspective of health economics.

Biomarkers within the blood and urine can reflect the status and possible future progression of a disease. As indicators of normal biological and pathological processes and as pharmacological indicators of therapeutic interventions, biomarkers allow measurements and evaluations to be made objectively (Gharbi et al., 2011). Currently, biomarkers are utilized clinically in the diagnosis of musculoskeletal diseases such as RA and osteoporosis. Moreover, biomarkers are used to monitor disease progression, make prognoses and evaluate the effectiveness of new drug treatments. Measurements of biomarkers in bodily fluids such as blood or urine are a simple means of evaluating a disease condition. In recent years, there has been a high demand for exploration of biomarkers for a variety of diseases (Kraus, 2011).

Recently, an increasing number of studies have investigated the diagnosis and prognosis of OA by measuring biomarkers present in the blood serum, urine, or synovial fluid of OA patients (Kraus, 2011). Biomarkers allow diagnosis of OA in early stages when macroscopic changes are few and difficult to detect, which enables highly objective diagnoses, compared to diagnoses using radiography; and provides quantitative evaluation of disease severity. Any protein with a differential expression between regular healthy cartilage and OA cartilage is a candidate diagnostic marker of OA. Cartilage oligomeric matrix protein (COMP) has been studied as a diagnostic marker, because it is a cartilage matrix component that is released into the blood and urine during cartilage degeneration (Kato et al., 2005). Fragments of C-terminal cross-linked telopeptide type II collagen (CTX-II), type II procollagen carboxy-propeptide (CPII), type II...
collagen-related neoepitope (C2C) and hyaluronic acid (HA) are examples of other possible candidates of diagnostic markers for OA. CTX-II and C2C are products of sequential degradation by several proteases originating from the cartilage matrix, including matrix metalloproteinase (MMP)-1, MMP-8 and MMP-13 (Comrozier et al., 2012).

Proteomic analysis is a research method to catalog all proteins inside cells and organisms. This method can elucidate protein structure and function as well as protein interactions inside the cell. In the general method of proteomics, samples are electrophoresed and the differential proteins are picked up as spots. After trypsin digestion, proteins are analyzed by mass spectrometry (Chambers et al., 2000). Proteins that are not included in spot areas cannot be detected. Furthermore, detected proteins with a low abundance cannot be used in quantitative analysis by conventional methods.

The isobaric tags for the relative and absolute quantitation (iTRAQ) method allow a more comprehensive analysis. Whole samples undergo trypsin digestion and then labeling with an iTRAQ reagent for analysis by mass spectrometry based on shotgun proteomics (Aggarwal et al., 2006; Ross et al., 2004). More than two digested samples can be labeled with separate reagents to perform quantitative analysis. This method has a high sensitivity and it is possible to detect low-abundance protein.

General proteomic analysis frequently identifies abundant proteins (ribosomal, proteasomal and cytoskeletal proteins as well as molecular chaperons) as biomarkers. However, it is well known that rare proteins such as secretory proteins and kinases are also important for various biological functions. In this study, among the lysates eluted through a random peptide column, we detected molecular variations in low-abundance proteins that were difficult to identify by conventional methods.

Proteomic analysis of serum from OA patients has revealed increased levels of the C-terminal end product of the V65 vitronectin subunit, C3f peptide, and connective tissue-activating peptide III (CTAPIII) (de Seny et al., 2011), as well as HPT (Fernandez-Costa et al., 2012), COMP, apolipoprotein, haptoglobin precursor, calgranulins, defensins and thymosins have been detected in synovial tissue (Kong, 2012; Kriegsmann et al., 2012). Furthermore, OA cartilages were electrophoresed and the differential proteins are picked up inside the cell. In the general method of proteomics, samples are electrophoresed and the differential proteins are picked up as spots. After trypsin digestion, proteins are analyzed by mass spectrometry (Chambers et al., 2000). Proteins that are not included in spot areas cannot be detected. Furthermore, detected proteins with a low abundance cannot be used in quantitative analysis by conventional methods.

Patients and articular cartilage
Control samples were obtained from 6 patients with femoral neck fracture (4 male and 2 female patients). The samples were collected from femoral head cartilage during bipolar femoral head replacement at Fujita Health University Hospital. OA cartilage samples were obtained from 17 patients with OA (5 male and 12 female patients). These samples were collected from the femoral heads, femoral condyles, and tibial plateaus of patients with primary OA, who underwent total hip or knee replacement surgery at Fujita Health University Hospital (Table 2).

Protein preparation
Samples were snap frozen at −80°C and cut into 10 μm sections by using a cryo-microtome (CM1900; Leica, Grove, IL). The sectioned samples were homogenized by sonication (T10 basic Disperser/Homogenizer; IKA) in RIPA buffer (10 mL of 50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% NP40, 0.5% sodium deoxycholate and 0.1% SDS/g cartilage). Then, the samples were centrifuged at 20 000 × g at 4°C for 10 min, and the supernatant was used for analysis. Amicon Ultra Centrifuge Filters 10 K (Millipore, Billerica, MA) were used to concentrate proteins by centrifugation at 5000 × g. Concentrated samples were stored at −80°C until use. A random peptide column (Proteominer; BIO-RAD, Hercules, CA) was used to prepare samples for iTRAQ. High-abundance proteins in these samples were excluded, while rare proteins were collected. Eluted samples were concentrated by Amicon Ultra Centrifuge Filters 10 K by centrifugation at 14 000 × g.

iTRAQ method
The protein samples were buffer exchanged and concentrated using 4-mL spin concentrators with a 5-kDa molecular weight cut-off according to supplied protocols (Agilent Technology, Santa Clara, CA). Briefly, the samples were centrifuged at 2600 × g for 20–30 min at 4°C. The protein samples were then buffer exchanged in the appropriate buffer containing 0.5 M triethylammonium bicarbonate for further analysis. The protein concentration of each sample was measured using a BCA Protein Assay Kit (Pierce, Rockford, IL). From each sample, 100 μg of protein was reduced, alkylated, and digested prior to labeling with iTRAQ reagent according to the manufacturer’s instructions (AB Sciex, Framingham, MA). Digested proteins prepared from each sample were labeled with iTRAQ reagent, and then pooled and washed according to the manufacturer’s instructions (AB Sciex, Framingham, MA). Peptides were subjected to a trap and reverse-phase (RP) analytical column by using a gradient of 0–50% solvent B in solvent A over 140 min (solvent A: 0.1% trifluoroacetic acid [TFA] and 2% acetonitrile; solvent B: 0.1% TFA and 70% acetonitrile) and 50–100% solvent B for 5 min at a flow rate of 300 nL/min. The RP analytical column eluent was spotted onto a MALDI sample plate by using a DiNa Direct Nano-flow LC/MALDI system (KYA Tech, Tokyo, Japan) and analyzed by a 4800 mass spectrometer (AB Sciex, Framingham, MA). Relative protein abundance was determined using MS/MS scans of the iTRAQ-labeled

Materials and methods
This study was approved by the steering committee and conducted under the guidelines for clinical studies of Fujita Health University. Human cartilage from OA patients and control subjects was obtained with informed consent.
peptides. iTRAQ-labeled peptides were fragmented under collision-induced dissociation conditions to generate fragment ions that provided sequence information for the peptide and reporter ions. Thus, the identity of the protein from the analyzed peptide was confirmed, and the ratios of the peak areas of iTRAQ reporter ions were used to compare the relative abundance of the protein identified in the sample. ProteinPilot v4.0 (AB ScieX, Framingham, MA) was used for data analysis using NCBI and SWISS-PROT databases.

Western blot analysis

Protein samples (35 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred onto polyvinylidene difluoride membranes. The membranes were blocked in 10% goat serum for 1 h at room temperature. Then, the membranes were probed with anti-LECT2 (ab89835, 1/250 dilution; Abcam), or anti-BAALC (H00079870-M01, 1/100 dilution; Abnova) antibodies for 1 h at room temperature, followed by incubation with a horseradish peroxidase-conjugated secondary antibody, and then chemiluminescence detection by ECL plus. Captured images were analyzed using a LAS4000 (Fuji Film) and Multi Gauge v2.0 (Fuji Film). For the anti-PRDX6 (MAB3490, 1/250 dilution; R&D SYSTEMS) antibody, the membrane was blocked in 5% skim milk. An XL-SAP Kit (APRO Science) was used for secondary antibody reactions. As internal controls, 35 μg protein samples (control: n = 5; OA: n = 16) were separated by SDS-PAGE, and then, SYPRO ruby protein gel staining (Invitrogen, Carlsbad, CA) was performed according to the manufacturer’s instructions. Gel images were taken by a Typhoon scanner (GE Healthcare, Tyrone, PA). Total intensity measurements of 10–200 kDa proteins were analyzed by image analysis software (ImageJ, Wayne Rasband (NIH), DC). For quantification of western blot data, each protein signal was quantitated and normalized by the total protein intensity.

Kellgren–Lawrence grade (Kellgren & Lawrence, 1957)

Grade 0: normal
Grade 1: doubtful narrowing of the joint space and possible osteophytic lipping
Grade 2: definite osteophytes and definite narrowing of the joint space
Grade 3: moderate multiple osteophytes, definite narrowing of the joint space, and some sclerosis and possible deformity of the bone contour
Grade 4: large osteophytes, marked narrowing of the joint space, and severe sclerosis and definite deformity of the bone contour.

Results

For the iTRAQ method, a control sample was obtained from a patient with femoral neck fracture (female, 46 years old). An OA sample was also obtained from a patient with hip OA (female, 59 years old). These samples were collected during arthroplastic surgeries at Fujita Health University Hospital.

Before applying the iTRAQ method, we used random peptide columns (BIO-RAD, Hercules, CA, Proteominer) to concentrate rare proteins in extracts from control and OA cartilages. Eluted proteins from random peptide columns were then analyzed by iTRAQ (Figure 1A).

We next investigated whether the expression levels of LECT2 (leukocyte cell-derived chemotaxin-2) were significantly higher in OA cartilage (LECT2: control, n = 5; OA, n = 16; p = 0.005). BAALC (brain and acute leukemia, cytoplasmic) expression was increased in OA cartilage compared with that in control cartilage (BAALC: control, n = 5; OA, n = 16; p = 0.051). PRDX6 (peroxiredoxin-6) expression was significantly reduced in OA cartilage compared with that in controls (PRDX6: control, n = 5; OA, n = 16; p = 0.011) (Figure 2A and B).

We next investigated whether the expression levels of LECT2, BAALC, and PRDX6 proteins in OA patients were associated with the trends in each parameter, including age, Kellgren–Lawrence grades, height, body mass index (BMI), gender, and joint site (Figure 3A–F). People over 65 years of age were defined as elderly in Japan. There was a significant difference in age and LECT2 expression in patients over 65 years.
years of age \((p = 0.0012)\). BAALC and PRDX6 showed almost no difference in expression (Figure 3A). In terms of Kellgren–Lawrence grade, there were almost no differences in LECT2, BAALC and PRDX6 expression (Figure 3B). To compare height, we divided the patients into groups of >160 cm or <160 cm. According to the annual health report of the Ministry of Health, Labour and Welfare of Japan in 2011, 160 cm was considered as the standard height for men.

### Table 1. The articular cartilage proteome.

| No. | Accession | Name                                         | Ratio of OA to control |
|-----|-----------|----------------------------------------------|------------------------|
| 1   | gi179665  | Complement component C3                     | 2.681                  |
| 2   | gi119605141| Cartilage oligomeric matrix protein, isoform CRA_a | 2.391                  |
| 3   | gi158257468| Unnamed protein product                      | 1.911                  |
| 4   | gi158255148| Unnamed protein product                      | 1.821                  |
| 5   | gi1000745  | Pro-a2(XI)                                   | 1.791                  |
| 6   | gi62205353 | Matrix Gla protein                           | 1.758                  |
| 7   | gi193787240| Unnamed protein product                      | 1.756                  |
| 8   | gi118573093| Ovochymase-1                                 | 1.656                  |
| 9   | gi4220894  | Transcriptional co-activator CRSP70          | 1.652                  |
| 10  | gi50295448 | Platelet phospholipase A2                    | 1.633                  |
| 11  | gi194377958| Unnamed protein product                      | 1.632                  |
| 12  | gi11920552 | Protein sidekick-1 precursor                 | 1.623                  |
| 13  | gi119604277| Pleiotrophin                                 | 1.596                  |
| 14  | gi11987667 | hCG2045237                                   | 1.573                  |
| 15  | gi940106   | T-cell receptor delta chain                  | 1.571                  |
| 16  | gi33464882 | Hypothetical protein                         | 1.527                  |
| 17  | gi49113217 | HHIP-like 2                                  | 1.524                  |
| 18  | gi348041283| C-type lectin domain family 3 member A isoform 1 | 1.514                  |
| 19  | gi62420929 | Actin-like protein                           | 1.503                  |
| 20  | gi18763987 | PRSS3 protein                                | 1.471                  |
| 21  | gi193786062| Unnamed protein product                      | 1.435                  |
| 22  | gi18307851 | Angiogenin                                   | 1.433                  |
| 23  | gi62897225 | Transforming growth factor, beta-induced, 68 kDa variant | 1.425                  |
| 24  | gi68563369 | Solute carrier family 4, anion exchanger, member 1 | 1.414                  |
| 25  | gi21757045 | Unnamed protein product                      | 1.411                  |
| 26  | gi59806345 | Leukocyte cell-derived chemotaxin-2 precursor | 1.389                  |
| 27  | gi1017427  | Elastic titin                                 | 1.381                  |
| 28  | gi119609838| Phosphatidylserine receptor, isoform CRA_d    | 1.376                  |
| 29  | gi609342   | Nucleophosmin-anaplastic lymphoma kinase fusion protein | 1.369                  |
| 30  | gi21755876 | Unnamed protein product                      | 1.341                  |

The top 30 proteins that were increased or decreased in OA cartilage on iTRAQ method were extracted.
Table 2. Characteristics of the OA patients and controls in this study.

| No. | Sample | Age | Gender | Height | Weight | BMI  | K-L Grade | Joint |
|-----|--------|-----|--------|--------|--------|------|-----------|-------|
| A   | Patients list that was used in the iTRAQ analysis | | | | | | | |
| 1   | Control | 46  | F       | 156    | 55     | 23   | –         | –     |
| 2   | OA     | 59  | F       | 150    | 51     | 23   | IV        | Hip   |
| B   | Patients list that was used in the Western Blot analysis | | | | | | | |
| 1   | Control | 70  | M       | 165    | 32     | 12   | –         | –     |
| 2   | OA     | 50  | F       | 156    | 51     | 23   | IV        | Hip   |
| 3   | Control | 31  | F       | 162    | 46     | 17   | –         | –     |
| 4   | OA     | 39  | F       | 180    | 81.5   | 25   | –         | –     |
| 5   | Control | 77  | M       | 164    | 52     | 19   | –         | –     |
| 6   | OA     | 75  | M       | 159.5  | 70     | 27   | III       | Hip   |
| 7   | OA     | 60  | F       | 153    | 49     | 20   | III       | Hip   |
| 8   | OA     | 56  | F       | 156.8  | 56.2   | 22   | IV        | Hip   |
| 9   | OA     | 52  | M       | 173    | 73.8   | 24   | III       | Hip   |
| 10  | OA     | 63  | F       | 159    | 59     | 23   | IV        | Hip   |
| 11  | OA     | 67  | F       | 165    | 62.5   | 22   | III       | Knee  |
| 12  | OA     | 74  | F       | 147.8  | 54     | 24   | IV        | Knee  |
| 13  | OA     | 87  | F       | 151    | 50     | 21   | IV        | Knee  |
| 14  | OA     | 82  | F       | 135    | 49.7   | 27   | IV        | Knee  |
| 15  | OA     | 79  | F       | 143.7  | 37.7   | 18   | III       | Knee  |
| 16  | OA     | 76  | M       | 152    | 52     | 22   | IV        | Knee  |
| 17  | OA     | 75  | F       | 153    | 57.4   | 24   | IV        | Knee  |
| 18  | OA     | 77  | F       | 146.5  | 64     | 29   | III       | Knee  |
| 19  | OA     | 63  | M       | 162    | 69.1   | 26   | III       | Knee  |
| 20  | OA     | 77  | M       | 174.3  | 59.7   | 19   | III       | Knee  |
| 21  | OA     | 81  | F       | 149    | 50.3   | 22   | II        | Knee  |

Figure 2. Western blot analysis of LECT2, BAALC and PRDX6. (A) We confirmed the protein expression levels of LECT2, BAALC and PRDX6 in OA and control. Protein gel was stained with SYPRO ruby. (B) Statistical analysis of western blot. Quantities are indicated relative to in OA and control. Protein gel was stained with SYPRO ruby. (B) confirmed the protein expression levels of LECT2, BAALC and PRDX6 in OA and control. Protein gel was stained with SYPRO ruby.

Discussion

In this study, we identified three new molecules that show expression differences between patients with OA and those with femoral neck fracture.

LECT2 is a neutrophil chemotactic factor expressed mainly in the liver (Yamagoe et al., 1996). It is a multifunctional protein involved in liver regeneration and immunocompetence. LECT2 is an effector of β-catenin-induced hepatitis that can progress to a precancerous lesion of hepatocellular carcinoma (Anson et al., 2012). A correlation has been observed between vascular invasion of hepatocellular carcinomas and a decline in LECT2 expression (Ong et al., 2011). There is a link between the disease severity of RA and LECT2 polymorphism in Japan (Kameoka et al., 2000), and LECT2 is an inhibitor of collagen-induced arthritis in mice (Okamura et al., 2008). LECT2 is also present in cartilage and it is identical to chondromodulin-II that has been purified from bovine epiphyseal cartilage. Moreover, LECT2 stimulates chondrocyte growth and matrix formation in vitro (Hiraki et al., 1996; Shukunami et al., 1999). In the present study, a significant increase in LECT2 expression was observed in OA cartilage (p = 0.005).

The receptor for LECT2 is C-type lectin DC-SIGN (CD209), an antigen receptor expressed in dendritic cells (DCs) of the skin, mucosal tissues and lymphatic tissues such as the tonsils, lymph nodes and spleen. It is also expressed in monocyte-induced DCs (Chen et al., 2010a). CD209 is highly expressed in inflammatory cells of the synovial membrane in RA patients, and has been found in the synovial membrane of OA patients (van Lent et al., 2003). The expression of CD209 depends on interleukin-4, and is inhibited by interferon, transforming growth factor-β, and anti-inflammatory drugs (Rellosa et al., 2002). These observations indicate an involvement of LECT2 in the pathophysiology of OA and RA.

The Wnt/β-catenin pathway is known to positively regulate LECT2 expression in the liver (Ovejero et al., 2004). An OA-like phenotype is induced by increased expression of the β-catenin gene in the cartilage of mouse models, suggesting that the Wnt/β-catenin pathway is associated with OA (Wu et al., 2010). Therefore, an association between increased LECT2 expression in OA and the Wnt/β-catenin pathway is possible. However, iTRAQ performed in the present study did not detect Wnt or β-catenin proteins. Therefore, the cause of the variation between control and OA groups could not be determined.
When the expression level of LECT2 was compared against various parameters in OA patients, LECT2 levels were found to be increased significantly in patients over 65 years of age ($p = 0.012$). When considering patients over 160 cm in height ($p = 0.057$), no significant difference in LECT2 levels was found, but a tendency toward increased expression was apparent. Although no correlation was observed among height, age, and different OA biomarkers, a relationship has been suggested between GDF5, which has been associated with OA, and height in humans (Williams et al., 2011). Therefore, the level of LECT2 may rise according to age. For this reason, we recalculated the expression level of LECT2 in OA and control cartilages by excluding cases in which the patients were under 65 years of age. As a result, we found significant up-regulation of LECT2 expression in the OA group compared with that in the control (Figure 4).

BAALC includes 6 isoforms and is abundant in acute myelogenous leukemia (AML) cells and the brain. Currently, it is being studied extensively as a diagnostic or prognostic marker for AML. There have also been reports of a correlation between BAALC and AML prognosis (Langer et al., 2008). Histone post-translational modifications such as H3K9K14 acetylation, H3K4 trimethylation and H3K23 trimethylation are involved in the regulation of BAALC expression in AML cells (Franzoni et al., 2012). In animal models, inhibitory effects on OA have been observed after intra-articular injection of trichostatin A, an inhibitor of histone deacetylase (Chen et al., 2010b). These observations show that cartilage cell degeneration might lead to epigenetic changes, resulting in altered BAALC levels in OA patients. Thus, OA prognosis and its correlation with BAALC are areas of current interest.

Although there have been no previous studies regarding the relationship between BAALC and OA, a study has shown variation in microRNA abundance based on differences in weight loads for each layer of articular cartilage in a bovine model. Non-weight-bearing regions show downregulated expression of miR-148a, a microRNA that is associated with leukemia, compared with the expression level in weight-bearing regions (Dunn et al., 2009). miR-148a expression shows a negative correlation with BAALC gene expression (Langer et al., 2008), and high BAALC gene expression correlates with the presence of an early-stage marker of precursor cells. This observation suggests that cell proliferation is stimulated in weight-bearing regions of articular cartilage. Therefore, an increase in the amount of BAALC in OA patients ($p = 0.051$) could modulate cell proliferation in damaged cartilage.
PRDX6 is an enzyme that has both glutathione peroxidase and lysosomal-type phospholipase A2 activities, and also possesses an antioxidant activity. The link between OA and ROS is well known with ROS increasing in OA patients (Koorts et al., 2012; Xie et al., 2012). The expression of PRDX5, which belongs to the same peroxiredoxin family, increased significantly in OA cartilage. In other studies, the expression of PRDX5 has been suggested to increase as a protective measure against tissue damage caused by ROS (Wang et al., 2002).

In a proteomic analysis of cartilage extracts from mice, PRDX6 was detected, but the correlation between PRDX6 and OA was not studied (Wilson et al., 2008). However, we found that the amount of PRDX6 was significantly reduced in OA cartilage, compared with the control group, suggesting that protection against tissue damage from ROS by PRDX6 is prevented in OA. Taken together, these results strongly suggest that PRDX family members are involved in protection against damage caused to cartilage by ROS, and that these proteins may be applicable in therapeutic intervention. Our study also identified the utility of PRDX6 as a marker. No correlation was found between PRDX6 abundance and age, height, BMI, gender or Kellgren–Lawrence grade.

Based on previous studies, a correlation between OA and BMI is well known. The OA risk factor is a BMI of $\geq 30$ kg/m$^2$ in WHO research (Gudbergsen et al., 2013; Wills et al., 2012). However, average weight and BMI in Japan are low compared with those in Europe and the United States (WHO expert consultation, 2004). In our current study, average weight and BMI were 56 kg and 22 kg/m$^2$, respectively. These values are obviously lower than the worldwide average. In addition, there was no correlation between OA and BMI because of the low number of patients with high BMI in the current study. For these reasons, we do not deny the correlation between OA and BMI.

In addition to cartilage-specific molecules such as CTX-II, CPII, C2C, HA, MMP-1, MMP-8 and MMP-13 (Conrozier et al., 2012), non-organ specific molecules such as fibulin-3 peptides (Henrotin et al., 2012), follistatin-like protein 1 (Wang et al., 2011b), and C3 (Wang et al., 2011a) are useful as OA biomarkers that can reflect OA pathology. In our current study, LECT2, BAALC and PRDX6 were not cartilage-specific proteins. Considering the above reports, we believe that these proteins may also be useful as OA biomarkers.

Because serum biomarkers are easy to measure continuously, they are considered useful for prognostication of OA. Furthermore, combined with diagnostic imaging results, the results of our study may assist OA diagnosis. LECT2 is a secreted protein that can be detected in serum (Sato et al., 2004). BAALC can also be detected in serum (Baldus et al., 2003). Although PRDX6 is also detected in serum, it is an enzyme and not a secreted protein (Zhang et al., 2009). As mentioned above, there is a high possibility that these proteins can be detected and measured in serum samples from OA patients. Therefore, they may be useful biomarkers for OA. As a next step, it will be important to perform large-scale research using serum from OA patients.

Of the previously identified OA markers such as CTX-II, C2C, COMP and HA, many are catabolic enzymes or fragments generated by proteolysis and/or degradation of the extracellular matrix. In addition to identifying COMP in OA cartilage, we confirmed variation in the expression of a molecule that could potentially function via autocrine or paracrine mechanisms in cartilage cells (LECT2), a molecule that could potentially function within cartilage cells (BAALC), and a molecule that has an antioxidant activity (PRDX6).

Conclusions

In developed countries with an aging population, the number of individuals with OA is predicted to continue to rise. Given the sheer number of such individuals, the utility of a marker that can be easily measured in blood, urine or synovial fluids cannot be ignored. In the future, efficient selection of patients with a high potential risk for progressive joint destruction and application of intensive prevention measures may drastically reduce the number of patients with severe joint destruction. To this end, identification and evaluation of an easily measurable biomarker is imperative. Combination of conventional markers with newly identified markers, the variation of which was confirmed in this study, may improve diagnosis of the OA disease state and the capacity for prognosis. We believe that it is necessary to perform large-scale clinical research using serum from OA patients to investigate useful biomarkers in the near future.

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Contributions

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version for publication.

Dr Yamada had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Ikeda, Ageta.

Acquisition of data. Ikeda, Ageta

Analysis and interpretation of data. Ikeda, Ageta.

Manuscript preparation. Ikeda, Ageta, Tsuchida, Yamada

Statistical analysis. Ikeda, Ageta.

Declaration of interest

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