The evolving role of biomarkers for osteoarthritis

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Abstract: Osteoarthritis (OA) is an increasingly important public health concern as the prevalence of this disease becomes higher and higher due to the ageing population. However, in addition to the absence of disease-modifying treatments, there are no sensitive diagnostic techniques beyond classical radiography, and physicians cannot predict who will progress with the disease. As a result, disease progression cannot be prevented or halted. Therefore, there is an urgent need for more effective techniques than radiography. Reliable, quantitative and dynamic tests to detect early damage and measure the progress of treatments targeted against joint destruction are required. Biomarkers, in addition to magnetic resonance imaging, are tools that can address these therapeutic shortcomings. Structural molecules and fragments derived from bone, cartilage and the synovium, all of which are affected by OA, have been reported to be potential candidates for biomarkers of OA. As the identification of biomarkers that can be applied more broadly from the very early to the end stages of knee OA is required, advances in the OA biomarker field remain challenging, but steadily progressive. Such advances will come not only from basic, but also preclinical and clinical research. In this review, we highlight recent OA biomarker studies generally published between 2011 and 2012. We classified the studies in this review into the following three categories: unique characteristics of the urinary level of C-terminal telopeptide of type II collagen; insight into the pathophysiology of OA revealed by biochemical biomarkers; and candidates for novel biomarkers of OA revealed by proteomics.

Keywords: osteoarthritis, biomarkers, CTX-II

Introduction

Osteoarthritis (OA) of the knee is a common joint disease in adults. Due to the significant growth of the ageing society, the number of patients with painful knee OA is dramatically increasing: the estimated number of patients is more than 25 million in the United States and 8 million in Japan [Muraki *et al.* 2009; Attur *et al.* 2011]. Routine radiography is an insensitive measurement of the molecular changes that occur in OA; therefore, predicting disease progression, such as cartilage degradation, joint space narrowing, osteophyte formation, subchondral sclerosis and bone marrow abnormalities, is challenging [Attur *et al.* 2013]. Hence, there is an urgent need to discover biochemical and imaging biomarkers that can be used to identify patients at risk for the progression of the disease, classify the molecular events that detect early changes of the disease and act as clinical surrogates that can be used to evaluate the response to such potential disease-modifying OA drugs (DMOADs) [Kraus *et al.* 2011]. In order to prevent disease progression and discover new DMOADs, clinicians must change their perception of the disease [Kraus *et al.* 2010]. Advances in the OA biomarker field remain challenging, but steadily progressive. Such advances will come not only from basic, but also preclinical and clinical research. In this review, we highlight recent OA biomarker studies published in 2011 and 2012 with our special interests. We classified the studies in this article into the following three categories: unique characteristics of the urinary level of C-terminal telopeptide of type II collagen (uCTX-II); insight into the pathophysiology of OA revealed by biochemical biomarkers; and, candidates for novel biomarkers of OA revealed by proteomics (Table 1).

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Table 1. Three categories of biomarker studies introduced in this review.

	Biomarkers	Samples	Expression	References
Unique characteristics of CTX-II	CTX-II	Urine	Cartilage, subchondral bone, osteophyte	van Spil *et al.* [2013]
				van Spil *et al.* [2012]
Insight into the pathophysiology of OA revealed by biochemical biomarkers	UA	Joint fluid	Inflammation	Denoble *et al.* [2011]
	n-6 / n-3 PUFAs	Plasma	Synovitis	Baker *et al.* [2012]
	COMP	Serum	Cartilage	Erhart-Hledik *et al.* [2012]
	Adipokines	Serum	Inflammation	van Spil *et al.* [2012]
Candidates for novel biomarkers in OA revealed by proteomics	Hemopexin	Serum	Glycosylation	Fukuda *et al.* [2012]
	Clusterin	Serum		
	AGP-2	Serum		
	MSP	Serum		
	Fib3-1, Fib3-2	Urine	Cartilage	Henrotin *et al.* [2012]

AGP-2, α1 acid glycoprotein-2; COMP, cartilage oligometric matrix protein; CTX-II, C-terminal telopeptides of type II collagen; Fib, fibulin; MSP, macrophage stimulating protein; N-6/N-3 PUFAs, ω6/ω3 polyunsaturated fatty acids; OA, osteoarthritis; UA, uric acid.

Unique characteristics of uCTX-II

A CTX-II was discovered to be a marker of collagen type II degradation [Christgau *et al.* 2001]. The uCTX-II has been shown to be useful as a diagnostic biomarker [Christgau *et al.* 2001; Jung *et al.* 2004; Reijman *et al.* 2004] and prognostic biomarker [Reijman *et al.* 2004; Sharif *et al.* 2007], while also being useful as a biomarker for evaluating the efficacy of treatment for knee OA [Conrozier *et al.* 2011]. However, the unique expression pattern of CTX-II in the joints [Bay-Jensen *et al.* 2008] and the unique association between the uCTX-II level and metabolic bone biomarkers [Bingham *et al.* 2006] has raised the question of whether or not uCTX-II levels are not solely a cartilage degradation biomarker.

A study by van Spil and colleagues challenged the use of the uCTX-II level as a cartilage degradation biomarker using the database of the Cohort Hip and Cohort Knee (CHECK) study [van Spil *et al.* 2013]. In this study, a long-term follow-up cohort of 1002 participants were included who were between 45 and 65 years of age at the time of inclusion with pain or stiffness of one or both knees or hips and who had never visited a general physician for these complaints, or had visited a general physician for the first time no longer than 6 months earlier. At baseline, the participants (79.0% women) were 56 years old on average and had a body mass index (BMI) of 25.5 kg/m². At baseline, 68% and 79% of the participants exhibited a maximum Kellgren and Lawrence (K/L) grade of 0, and 25% and 15% exhibited a grade of 1 for knees and hips respectively. The subjects were assessed using Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) subscales scores and several biomarkers, including uCTX-II, the cartilage degradation marker serum cartilage oligometric matrix protein (sCOMP), the cartilage synthesis markers type IIA collagen N-propeptide (sPIIANP) and chondroitin sulfate 846 (sCS846), the bone degradation markers N-terminal telopeptides of type I collagen (uNTX-I) and C-terminal telopeptide of type I collagen (uCTX-I) and the bone synthesis markers aminoterminal propeptide of type I procollagen (sPINP) and osteocalcin (sOC), were evaluated. The biomarkers of bone degradation were moderately to strongly associated with those of bone synthesis. Notably, the uCTX-II level exhibited weak to moderate positive associations with all bone markers and very weak, at best, associations with the other cartilage markers. The bone degradation markers were more strongly associated with the uCTX-II level than the bone synthesis markers. In comparison, associations between the other cartilage markers and the bone markers were less evident. In addition, the uCTX-II levels demonstrated a postmenopausal increase in women similar to that observed in the bone markers, in contrast to the cartilage markers.

The authors also reported the findings of another study using the CHECK database [van Spil *et al.* 2012a]. They assessed a wide spectrum of OA-related biomarkers in the CHECK database of individuals with early symptomatic knee or hip OA. The authors investigated the associations between the biomarkers and the demographics of the patients in order to demonstrate the validity of the obtained dataset and further investigate the involvement and role of these biomarkers in OA.

A principal component analysis enabled the identification of clusters of interrelated biomarkers within the biomarker spectrum. The authors also focused on the uCTX-II level. The 'bone-CTX-II' contained not only bone markers but also the uCTX-II level. As discussed previously, the uCTX-II level has been suggested to be primarily derived from osteoclastic resorption of calcified cartilage, and the localization of the CTX-II epitope occurs at the cartilage–bone interface [Bay-Jensen *et al.* 2008].

It has been reported that the uCTX-II levels in patients with knee OA exhibit stronger associations with the levels of osteophytes than with joint space narrowing [Jordan *et al.* 2006; Davis *et al.* 2007]. Positive associations have also been reported between uCTX-II levels and the NTX-I and CTX-I levels in postmenopausal patients with osteoporosis with and without OA [Tanishi *et al.* 2009; Karsdal *et al.* 2010; Kraus *et al.* 2010a]. Antiresorptive therapy with bisphosphonate primarily aimed at bone metabolism also decreases uCTX-II levels [Bingham *et al.* 2006]. However, the uCTX-II level does not exclusively reflect bone turnover. The associations between uCTX-II level and metabolic bone biomarkers are weaker than those observed between the bone metabolic biomarkers themselves. In addition, the uCTX-II level was positively associated with BMI, while bone metabolic biomarkers were negatively associated with BMI. The uCTX-II level has been shown to express the status of the borderline region between calcified cartilage and subchondral bone and is associated with osteophyte formation [Bay-Jensen *et al.* 2008].

Therefore, as uCTX-II exhibits distinct kinetics in comparison to other cartilage degradation markers, changes in the uCTX-II level may reflect the functional unit formed by the articular cartilage and subchondral bone, which is of particular interest when considering the pathogenesis of OA [Lories and Luyten, 2011]. Osteophytes are also

of particular interest as they have been revealed to be related to disabilities in daily life, while joint space narrowing is related to knee pain [Muraki *et al.* 2012]. The uCTX-II levels in subjects with K/L grade 2 in the presence of knee pain are significantly higher than those observed in subjects with K/L grade 2 in the presence of knee pain. However, in subjects with K/L grade 1, the uCTX-II levels are not changed by the presence of knee pain [Ishijima *et al.* 2011]. In addition, the uCTX-II level has been reported to be a predictive factor for the progression of knee OA [Sowers *et al.* 2009]. Based on these results, the uCTX-II level should receive further attention, as it is speculated to be more deeply involved in the pathogenesis of OA.

Insight into the pathophysiology of OA revealed by biochemical biomarkers

Uric acid (UA) activates the Nacht, leucine-rich repeat and pyrin domain containing protein 3 (NLRP3) inflammasome. When the NLRP3 is activated, the inflammasome stimulates the production of interleukin (IL)-18 and IL-1β. Denoble and colleagues hypothesized that UA in the knee joint regulates inflammation and the severity of knee OA [Denoble *et al.* 2011]. Among the 159 subjects of the Prediction of Osteoarthritis Progression (POP) study, the UA levels in the joint fluid of 69 subjects were measured. While the UA levels in the serum of the subjects were strongly and positively associated with those in the joint fluid, the UA levels in the joint fluid were also strongly and positively associated with both the IL-1β and IL-18 levels in the joint fluid. In addition, the UA levels in the joint fluid, similar to the IL-1β and IL-18 levels in the joint fluid, were independently associated with the osteophyte scores. These factors were also significantly and positively associated with the severity of OA evaluated on bone scintigraphy. Furthermore, the IL-1β and IL-18 levels in the joint fluid were associated with the changes in the osteophyte scores during the 3-year observation period. The results of this study suggest that the UA floating in the knee joint is associated with the severity of OA and may be deeply involved in the pathogenesis of knee OA via the actions of inflammasomes regulated by macrophages.

The role of synovitis in the pathogenesis of OA has attracted particular attention. The presence of synovitis in the pathogenesis of OA may be a secondary phenomenon related to cartilage and bone

alterations induced by the release of degenerative compounds from the extracellular matrix of articular cartilage in response to the presence of microcrystals in the synovial fluid and synovium [Ayral *et al.* 2005]. However, synovitis has been reported to be a potential predictive factor for both the structural and symptomatic progression of the disease [Ayral *et al.* 2005; Baker *et al.* 2010; Liu *et al.* 2010; Ning *et al.* 2011], thus indicating its crucial role in the pathophysiology of OA.

ω6 and ω3 polyunsaturated fatty acids (n-6 and n-3 PUFAs) are directly linked to inflammation via their role as precursors for a family of compounds known as eicosanoids. Eicosanoids are mediators and regulators of inflammation. Arachidonic acid (AA) is the primary n-6 PUFA found in inflammatory cells and induces the production of inflammatory eicosanoids. Studies have shown that an increasing amount of AA in the diet increases the content of AA in inflammatory cells and the production of inflammatory eicosanoids [Calder, 2006]. Higher levels of AA in the blood or diet have been tied to increased platelet aggregation and atherosclerosis in a subpopulation with a genetic variation in 5-lipoxygenase, an enzyme that generates inflammatory leukotrienes from AA [Dwyer *et al.* 2004]. In patients with rheumatoid arthritis (RA), a diet low in AA ameliorates the clinical signs of inflammation [Adam, 2003]. The n-3 PUFAs, eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), however, give rise to eicosanoids with a slightly different structure from those formed from AA. These eicosanoids are less potent mediators of inflammation. Treatment with n-3 PUFAs, predominantly EPA and DHA, results in lower levels of proinflammatory markers and higher levels of anti-inflammatory markers, and better outcomes in numerous diseases, including RA and cardiovascular disease [Balk *et al.* 2006, Ferrucci *et al.* 2006]. In addition, in cartilage cell cultures, n-3 PUFAs have been shown to inhibit the transcription of major enzymes and cytokines tied to matrix degradation [Zainal *et al.* 2009].

The 18:2n-6 and alpha linolenic acid (ALA; 18:3n-3), the precursors to AA, EPA and DHA, are essential in the diet. The predominant dietary n-6 PUFA is LA, which is converted to AA. In addition, some AA is obtained from meat consumption. The predominant dietary n-3 PUFA is ALA. It has been shown that only a very small portion is converted to the long chain n-3 EPA and DHA that appear to be the more potent inhibitors of inflammation. Sources of EPA and DHA in the diet include fatty fish, such as salmon, tuna, anchovies, sardines and shellfish, shrimp and Alaskan king crab. The current Western diet is higher in n-6 fatty acids with an n-6 to n-3 ratio of 20–30 to 1. This ratio has increased dramatically in recent years in association with increased food processing, reduced fish consumption and changes in the dietary habits of farm animals.

Baker and colleagues assessed the associations between the levels of fasting plasma phospholipid n-6 (AA) and n-3 PUFAs (EPA and DHA) and synovitis, as measured according to synovial thickening on contrast-enhanced magnetic resonance imaging (MRI) of the knee, among subjects enrolled in the Multicenter Osteoarthritis Study (MOST) [Baker *et al.* 2012]. Since cartilage loss on MRI may also reflect the influence of local inflammation, which may in turn be related to systemic inflammation [Baker *et al.* 2010], authors also examined the relationships between cartilage loss and the n-6 and n-3 PUFA levels. Among the 472 subjects (average 60 years of age, mean BMI 30, 50% women), half of the subjects exhibited radiographic knee OA and one-third demonstrated synovitis on MRI. No associations were observed between the levels of n-6 PUFAs and synovitis or cartilage loss. However, the higher quartiles of the n-6 PUFAs levels were associated with synovitis, not with cartilage loss. In addition, no associations were observed between the n-3 PUFA levels and synovitis. Higher levels of total n-3 PUFAs were associated with a reduced severity of patello-femoral cartilage loss, although they were not associated with tibio-femoral cartilage loss. Meanwhile, although the DHA level was not associated with synovitis, it was inversely associated with patello-femoral cartilage loss.

These data provide many suggestions to establish future strategies for treating OA. For instance, the availability of arachidonic acid for the production of inflammatory eicosanoids may be a predisposing factor for the development of synovitis in patients with OA, which is related to both the symptoms and progression of the disease [Ayral *et al.* 2005; Liu *et al.* 2010; Ishijima *et al.* 2011]. While little is known in the OA field, fish oil supplements have been used extensively as a component of combination treatment for RA [Cleland *et al.* 2006]. The benefits of ω3-rich supplements in patients with RA are well established, includ-

ing reduced severity of symptoms, increased rates of remission, improvements in markers of cardiovascular risks and the decreased discretionary use of nonsteroidal anti-inflammatory drugs [Cleland *et al.* 2006; Cleland and James, 2012]. The benefits of ω3-rich supplements may also be important potential advantages for patients with OA, although further research is required.

sCOMP is considered to be a potential predictive biomarker for knee OA [Hoch *et al.* 2011]. As sCOMP is mechano-sensitive and plays a role in transducing mechanical forces in the extracellular matrix to the cells [Wong *et al.* 1999], Erhart-Hledik and colleagues hypothesized that the use of a mechanical stimulus may be an effective approach for evaluating the sensitivity of COMP as a prognostic indicator of the health status of cartilage [Erhart-Hledik *et al.* 2012]. The authors planned to analyze the role of sCOMP in a stimulus–response framework, in which changes in the sCOMP concentration in response to a mechanical stimulus have the potential to achieve greater efficacy in detecting the underlying pathology of OA. Whether the sCOMP level induced in response to a mechanical stimulus, such as a 30 min walk, is associated with changes in cartilage thickness over a 5-year follow up was examined. The subjects (average 59.0 years of age, mean BMI 28.1 kg/m^2 and mean K/L grade 2.2) took 3527 steps at 114 steps/min while walking for 30 min. No associations were observed between the baseline sCOMP level and the degree of joint space narrowing of the knee joint over the 5-year follow up. The sCOMP levels were not significantly different from the preactivity levels 3.5 h after walking. However, the sCOMP levels were significantly reduced 5.5 h after walking. There were no associations between the changes in the sCOMP levels after exercise and either the number of steps taken or the distance walked during the 30 min walking exercise. Interestingly, there was a significant negative correlation between the changes in the COMP levels at 5.5 h post activity and the changes in cartilage thickness in the total medial femur (*p* = 0.029) and total medial tibia (*p* = 0.050). The authors speculated that the changes in the COMP levels following a mechanical stimulus are indicative of increased rates of cartilage degradation and appear to be caused by altered metabolic activity rather than diffusion due to walking. Although further studies are needed, the abnormal tissue metabolism observed in response to the walking exercise may indicate a greater disease activity, as

reflected by cartilage degradation 5 years later. Furthermore, this study provided several future suggestions and directions for OA biomarker research field.

Obesity, as well as aging, is a risk factor for the development of OA. Although the association between obesity and knee OA has been attributed to biomechanical mechanisms, a hormonal mechanism has been speculated to also be involved, as weight and BMI are associated with the development of hand osteoarthritis [Yusuf *et al.* 2010]. In one study, van Spil and colleagues evaluated adipokines, which are secreted from adipose tissue, to which a metabolic link between OA and BMI may be attributed [van Spil *et al.* 2012b]. Previous studies examining the relationship between OA and adipose tissue have shown inconsistent results [Honsawek and Chayanupatkul, 2010; Berry *et al.* 2011; Koskinen *et al.* 2011]. In this study, the levels of plasma adipokines were investigated for potential relationships with joint metabolism and OA in 1002 subjects with early-stage symptomatic knee or hip OA (CHECK study). The sLeptin levels were positively associated with the levels of uCTX-II and sCOMP, sPIIANP, sHA and sPIIINP following adjustment for sex and age. These associations expectedly disappeared following adjustment for BMI, except for the associations with the levels of sPIIANP and sPIIINP. The levels of pAdiponectin exhibited positive associations with the levels of uCTX-II and sCOMP, which became stronger after adjusting for BMI. The levels of pResistin demonstrated a weakly positive association with the levels of sPIIINP, which were not affected by adjusting for BMI. The levels of sLeptin were weakly associated with the presence and progression of radiographic knee OA and associated with the progression of OA. However, these associations disappeared after adjusting for BMI. In addition, the sLeptin levels were not associated with the incidence of knee OA. The sAdiponectin levels were not associated with the presence, incidence or progression of knee OA. The sLesistin levels were associated with the presence and incidence of knee OA; these associations were not affected by adjusting for BMI. The sLesistin levels were also not associated with the progression of knee OA.

When the interactions between adipokines were examined, no statistically significant interactions between the levels of adipokines and their associations with radiographic knee OA were observed, except for the levels of pResistin and

pAdiponectin, which exhibited an interaction in their association with the presence of radiographic knee OA (p = 0.024). When the association of pResistin with the presence of radiographic knee OA was tested per the pAdiponectin quartile, it appeared that the positive association between the pResistin levels and the presence of radiographic knee OA was observed only in the upper two pAdiponectin quartiles. Adiponectin is speculated to be involved in the development of inflammation. Adiponectin plays a proinflammatory role in OA based on its positive associations with the production of synovial IL-1β in patients with end-stage knee OA and the association between the highly sensitive C-reactive protein level and the synovial fluid adiponectin level in patients with knee OA [Schaffler *et al.* 2003; De Boer *et al.* 2012]. Relationships between the occurrence and progression of OA and risk factors of metabolic syndrome have been reported [Yoshimura *et al.* 2011, 2012], and the results of this study indicate the aggravating role of these adipokines in early-stage knee OA, although their major role has not been elucidated.

Candidates for novel biomarkers in OA revealed by proteomics

Proteomic analyses, based on mass spectrometry (MS), have been developed to explore biomarkers in a variety of scientific fields [Fernandez-Puente *et al.* 2011; Fukuda *et al.* 2012; Henrotin *et al.* 2012; Mateos *et al.* 2012]. Fukuda and colleagues identified four candidate molecules as potential prognostic biomarkers for knee OA using an N-glycoproteomic 2D-LC-MALDI analysis [Fukuda *et al.* 2012]. Serum samples and knee radiographs were obtained every 6 months from 68 subjects with primary knee OA during the 18-month study period. In that study, the progressors were defined as patients whose radiographic joint space width decreased continuously for 18 months or three consecutive 6-month periods. The nonprogressors were defined as patients whose radiographic joint space width did not show any detectable changes for three consecutive 6-month periods. Based on these definitions, three subjects were determined to be progressors, while 12 subjects were identified to be nonprogressors. Among the 12 nonprogressors, three age-, sex- and BMI-matched subjects were selected as controls. Serum samples were subjected to an N-glycoproteomic 2D-LC-MALDI analysis, and the glycoproteins represented by the four selected MS peaks were subjected to an MALDI-TOF/TOF MS analysis. Among the four identified proteins, a peptide fragment of G^{235}HGHRNGTGHGNSTHHGPEYMR^{256} was found to have originated from hemopexin, which was most strongly expressed in the progressors compared with that observed in the nonprogressors. A peptide fragment of L^{26}VPVPITNATLDRITGK^{42} was identified to be a peptide generated from α1 acid glycoprotein 2. The other two molecules were clusterin and macrophage stimulating protein.

In this study, the authors applied the glycoproteomic approach in order to discover biomarkers. The changes in the peak intensities reflected not only the change in the amount of a given protein but also the change in the level of glycosylation on that protein. With respect to hemopexin and clusterin, the concentrations did not differ significantly between the progressors and nonprogressors. The authors speculated that the level of glycosylation may increase during the progression of the disease, although further study is needed. While the precise molecular mechanisms of these molecules in the pathogenesis of knee OA remain unclear, the molecules are involved in inflammation. Recently, inflammation, particularly synovitis, has received attention in the OA research field. These four molecules not only play an important role as predictive biomarkers of OA, but also contribute to clarifying the pathogenesis of the disease.

Henrotin and colleagues explored novel biomarkers in urine samples using a proteomics analysis [Henrotin *et al.* 2012]. The urine samples were collected from 10 female patients with severe knee OA who underwent knee replacement surgery (average 76.0 years of age) and five healthy female controls (average 25.6 years of age) and subjected to a proteomics analysis. Protein spots that exhibited a ratio for OA according to a control of 1.5 or higher were identified using MS. Thirteen proteins were identified, and the authors were particularly interested in the two peptides of fibulin 3, named fibulin 3-1 (Fib3-1) and fibulin 3-2 (Fib3-2). Specific enzyme-linked immunosorbent assays were developed and validated in serum obtained from healthy subjects and patients with severe radiologic knee OA. The serum levels of Fib3-1 and Fib3-2 in the patients with OA were significantly higher than those observed in the age-matched healthy subjects (p < 0.0001). The area under the receiver operating characteristic curve indicated that the cutoff levels of serum

Fib3-1 and Fib3-2 corresponding to the presence or absence of radiographic knee OA were 71.15 pM (specificity 77.1%, sensitivity 68.4%) and 164.0 pM (specificity 85.7%, sensitivity 74.6%) respectively. An immunohistochemical analysis revealed that the Fib3-1 and Fib3-2 proteins were expressed in a thin layer of flattened chondrocytes at the cartilage surface in normal cartilage. In the OA cartilage with fibrillation, both molecules were strongly expressed in the extracellular matrix and cell clusters. The Fib3-1 and Fib3-2 proteins were not expressed in the inner zone of cartilage.

Fibulin 3 is a member of a family of extracellular matrix proteins characterized by tandem arrays of epidermal growth factor like domains and a C-terminal fibulin-type module. In adults, fibulin 3 is widely distributed in various types of tissues and blood vessels of different sizes and is capable of inhibiting vessel development and angiogenesis both *in vitro* and *in vivo* [Albig *et al.* 2006]. During development, fibulin 3 is expressed in the mesenchyme and mesenchymal tissues, such as cartilage and bone, and plays a role in organizing the development of the skeletal system. Fibulin 3 is intimately associated with tissue inhibitor of metalloproteinases 3. An overexpression of fibulin 3 in the clonal murine cell line ATDC5 negatively regulates chondrocyte differentiation [Wakabayashi *et al.* 2010]. The overexpression of fibulin 3 suppresses chondrocyte differentiation by inhibiting cartilage nodule formation, proteoglycan production and the matrix gene expression and selectively maintains the expression of SOX9 while suppressing the expression of SOX5 and SOX6. However, further studies are required to elucidate the role of fibulin 3, not only in the pathophysiology of OA, but also in the homeostasis of mature cartilage.

Conclusion

In addition to the biomarkers introduced in this review, genetic and epigenetic markers have also been noted recently. They have the potential to detect early changes or to prediagnose in OA and classify the OA itself as shown in cancers [Barter *et al.* 2012; Reynard and Loughlin, 2012].

It has recently been revealed in epidemiological research that the accumulation of metabolic syndrome components is significantly related to both the occurrence and progression of knee OA [Yoshimura *et al.* 2012]. The inflammatory properties of adipokines and their role in OA have also

been suggested and are described in this review [van Spil *et al.* 2012a, 2012b]. In order to prevent OA and discover effective DMOADs for this condition, clinicians must change their perception of the disease. Just as a heart attack or stroke can represent the end stages of long-simmering cardiovascular disease, radiographic joint space narrowing reflects joint failure that began to develop perhaps decades earlier [Kraus *et al.* 2010b]. What we call OA now is the outcome of a long, often silent disease process. OA must also be considered from a molecular point of view. There are many subjects to be evaluated. Most patients with knee OA do not need to undergo joint replacement, which is an option for patients with structurally or symptomatically severe knee OA. Moreover, most patients with radiographic knee OA spend their daily lives without any knee pain [Muraki *et al.* 2012]. It is important to consider OA a heterogeneous multicausal chronic joint disease syndrome to classify the disease in terms of both differences in pathophysiology and whether the patient is a progressor or nonprogressor. In addition, reduced mobility in older patients impairs activities of daily living. Joint replacement arthroplasty is an authorized alternative for patients with end-stage knee OA to maintain mobility. Undergoing joint replacement surgery is not an end point for patients with end-stage knee OA. Clinicians should take these factors into consideration when deciding whether a patient with end-stage knee OA should receive joint replacement surgery in order to maintain or obtain the ability to walk without pain [Kaneko *et al.* 2013; Shimura *et al.* 2013]. It is also necessary to discover effective DMOADs in future knee OA research. In order to achieve these long-term objectives, it is necessary to identify biomarkers of OA that can be applied more broadly from the very early stage to the end stage of knee OA and to design better management systems for patients with knee OA.

Conflict of interest statement

The authors declare no conflict of interest in preparing this article.

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