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Recent progress toward biomarker identification in osteoarthritis

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Osteoarthritis (OA), the most common and disabling form of arthritic disease, is characterized by a slow and progressive degeneration of articular cartilage. Its etiology is multifactorial and includes genetic predisposition, obesity and aging. In addition to the cartilage itself, OA also involves the surrounding tissues, including the synovium and the subchondral bone. This clinical heterogeneity complicates the identification of biomarkers that are crucial for prompt pharmacological intervention at the early stages of the disease and for monitoring treatment efficacy with higher sensitivity than existing imaging methods. In this review, we highlight the difficulties associated with OA diagnosis and discuss the most recent research efforts and successes for the identification of reliable OA biomarkers.

Introduction

In November 1999, the then General Secretary of the United Nations, Kofi Annan, declared the official endorsement of the Bone and Joint Decade (BJD) 2000–2010. In January 2000, the BJD was formally launched by the World Health Organization (WHO). Among several missions dedicated to improving the health-related quality of life for people with musculoskeletal disorders, one specific goal of the BJD was to achieve a 25% reduction in the expected increase in joint destruction in joint diseases (http://www.boneandjointdecade.org). Although the generic term 'joint disease' covers several rheumatic disorders, osteoarthritis (OA), also called degenerative joint disease, is by far the most widespread joint-affecting disease. It is estimated that it affects over 27 million people (or 12% of the total population) in the USA [1], compared with 0.6% for rheumatoid arthritis [2]. As the US population ages, nearly 67 million Americans could suffer from arthritis by the year 2030, with more than 50% of cases among adults older than 65 years [3]. Together with aging, the growing epidemic of obesity, an important risk factor for OA, and the sedentary way of life might further increase the prevalence of OA in the general population. Pain, stiffness and associated activity limitations are the main symptoms of OA. In addition to this major impact, the burden of OA is often worsened by a state of physical and mental fatigue accompanied by a reduction of the patient's social life and increased psychological distress [4,5]. In terms of socioeconomic costs, OA is a major public health concern. In France, the estimated direct annual costs of OA exceed €1.6 billion, corresponding to approximately 1.7% of expenses of the French Health Insurance system [6]. In Spain, the average total costs per patient are €1500 per year, and the national cost of OA is estimated to be €4.7 billion per year, representing 0.5% of the gross national product [7]. In the USA, taking into account out-of-pocket and insurer contributions, the medical care expenditures for OA are estimated at US$185 billion per year [8].

Regarding the increasing prevalence and burden of OA, multiple actions are urgently needed, including education campaigns for OA prevention, and funding research for the identification of treatments that address the causes rather than the symptoms of OA. Today, no drug has yet demonstrated any disease-modifying activity. Undoubtedly, the discovery of biochemical markers for early OA detection would help to identify new pharmacological treatments aiming at stopping OA before it becomes irreversible. In clinical practice, biochemical markers would enable physicians to monitor OA progression and assess treatment efficacy with more reliability than do the poorly sensitive and expensive imaging techniques that are currently used. The aim of this review is not to report an exhaustive list of the many OA biomarker candidates, but rather to address the question of why they are so important, with special emphasis on the following: the criteria that a biomarker candidate should fulfill for validation in OA

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prognosis and diagnosis; identification hurdles; and recent research studies giving reasons for hope.

Features of cartilage remodeling in OA

In healthy joints, the hyaline cartilage that normally covers the bone endings allows them to slide one over the other with little friction. In such cartilage, chondrocytes, the sole cell type present, are dispersed in a strongly hydrated extracellular matrix (ECM) that makes up more than 95% of the tissue volume. Water content is approximately 75% of tissue mass, and collagen is the most abundant organic component, approximately 75% of dry weight, followed by proteoglycans at 20%, the balance being mainly hyaluronan and small structural proteins.

Tissue structure is maintained through a balanced remodeling, carried out by chondrocytes, which are responsible for both the synthesis and degradation of the ECM. In osteoarthritic joints, articular cartilage undergoes episodes of catabolic events and attempts of repair, which lead first to disorganization and then a net loss of tissue. Over the course of these pathologic remodeling events, the chondrocytes fail both in number and function, with a switch to a mode favoring degradation over synthesis of ECM. It is possible to distinguish broadly different phases in OA development (Fig. 1). OA cartilage first enters a phase of synthetic activity, during which chondrocytes resume proliferation and produce an excess of ECM. In parallel, the subchondral bone can undergo significant remodeling. During the early progressive and often asymptomatic phase of the disease, increased bone resorption was documented [9]. Given that this attempt to repair apparently fails to re-establish structural and functional equilibrium, cartilage enters an early degradative phase in which, under the drive of cytokines such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNFα), or oncostatin M, chondrocytes produce enzymes (aggrecanases) that selectively target proteoglycans. The process is restricted to the superficial layer of articular cartilage and leads to the appearance of the first signs of fibrillation. A more intense degradative phase follows, carried out by matrix metalloproteinases (MMPs) that break up the collagenous scaffold and cause progressive erosion of cartilage, accompanied by synovial inflammation. Simultaneously, an increase in subchondral bone density is classically observed, associated with the formation of osteophytes.

The challenges of biochemical marker identification in OA

The identification of specific OA biomarkers is difficult, in part because of the clinical heterogeneity of this disease. Although

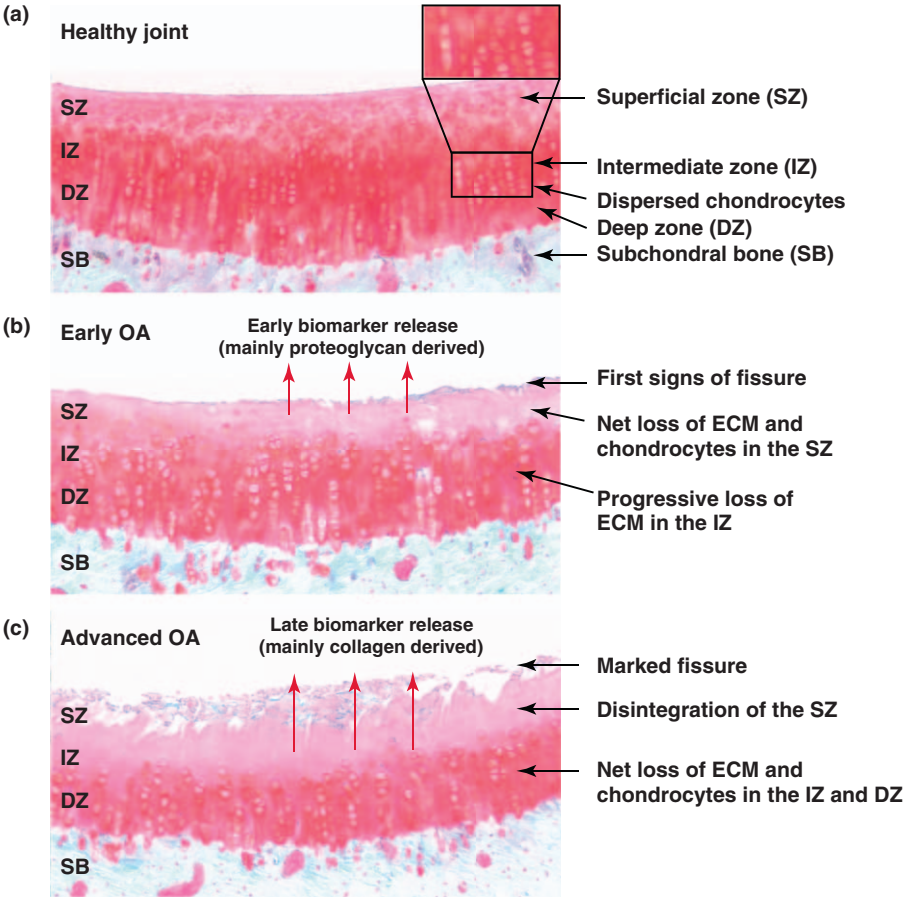

FIGURE 1

Schematic overview of the different phases of OA occurring in articular cartilage. **(a)–(c)** Histological slides illustrate the progressive loss of cartilage occurring in the tibial plateau of Hartley guinea pigs that develop OA spontaneously. OA in this guinea pig strain closely resembles that occurring in humans. Cartilage is colored red by safranin O staining. ECM, extracellular matrix.

serum and urine biomarkers for diagnosis and prognosis have been recently proposed, none can be used in clinical practice because of large interindividual variations. The progression of OA is slow and periodic, with intermittent episodes of inflammatory flares and remission periods, which can lead to the discontinuous release of potential markers in biological fluids. The early pathological events occurring at a molecular level in OA cartilage often appear several years before patients might experience the pain and stiffness that prompt consultation and formal diagnosis. The loss of articular cartilage in OA affects different joints in the body, including hands, knees, hips and spine, with a higher prevalence in women than in men [1–3]. As stated above, although remodeling events mainly occur in articular cartilage during OA, the synovium and the subchondral bone can also contribute to the release of OA biomarkers [10]. However, such identification remains challenging as bone and the synovium are also associated with prevalent diseases, such as osteoporosis or rheumatoid arthritis. By definition, a specific OA biomarker would miss its goal if it is simultaneously able to reflect other diseases. Similarly, inflammation markers intermittently produced during the course of OA should not be considered strictly as specific OA biomarkers because the inflammatory state is a hallmark of several other pathologies.

A second important consideration when defining an OA biomarker is the sampling site, which should be accessed easily for routine clinical use. Proteins or peptides generated during periods of cartilage remodeling in OA are first released into the synovial fluid (SF). Given that these molecules are at a higher concentration in the SF compared with other biological fluids, most studies looking for OA biomarkers have focused on the SF of the affected joints. However, because it is invasive, such a procedure would be largely impractical for large-scale clinical diagnosis and monitoring. In clinical practice, serum and urine are the biological fluids of choice for biomarker monitoring. In OA, potential biomarkers released into the SF during the course of cartilage remodeling are highly diluted in plasma and urine, and their identification requires sensitive and reliable techniques. Despite this additional hurdle, the growing awareness that biomarkers could help combat the burden of OA has prompted researchers to accept the challenge (Fig. 2).

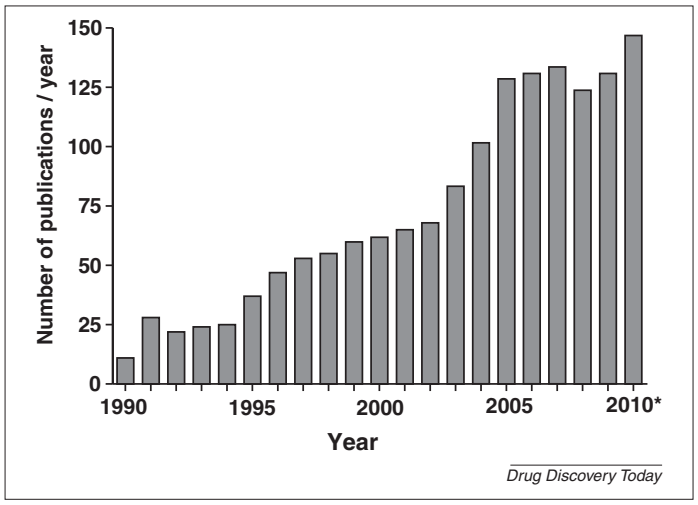

FIGURE 2

Number of publications relating to OA biomarkers during the period 1990–2010. The search was performed in PubMed for the two keywords 'Osteoarthritis' and 'biomarker'. For the year 2010, the search was performed with an endpoint of August 31. *The value given for 2010 is an estimate for the whole year calculated from the value obtained within this 8-month period.

Imaging markers as supports for biochemical marker identification

The current gold standard for diagnosing OA is X-ray, showing joint space narrowing (JSN), the formation of bony spurs (also called osteophytes) around the joint and the appearance of subchondral cysts. Despite their limited sensitivity, X-rays are indispensable support for the identification of biochemical markers. To illustrate this assertion, a panel of serum proteins implicated in cartilage matrix degradation, cell activation, inflammation and bone remodeling was recently proposed as possible biochemical markers of early OA, as a result of X-ray measurements made at ten-year intervals in a cohort of 88 initially healthy subjects [11]. Similarly, X-ray was a decisive parameter for qualifying two putative serum and urine OA biomarkers measured over a ten-year period as predictors of subsequent knee OA and stiffness [12]. Interestingly, a study in patients with clinical OA revealed that more adequate phenotyping based on X-ray measurements in several joints could be of help in the qualification of three candidate biochemical markers measured in serum or urine [13]. This study focused on the process of biomarker qualification for structural endpoints of OA (such as JSN and osteophytes) rather than on symptomatic endpoints (pain). Joint size was not found to be a major determinant of biomarker concentrations. With respect to concentrations of these three markers, X-ray features of the lumbar spine did not differ in appearance from those in other joints, suggesting that the biological processes occurring in lumbar spine were not different from those in other joints. Finally, the three biomarkers were found to be more powerful predictors of multijoint disease than of disease in a single joint. These results suggested that, upon adequate patient phenotyping, X-ray helps in the qualification of biomarkers in specific subspecies of OA and of total burden of the disease.

As cartilage is not visualized on radiographs, alternative techniques, based on the direct quantification of a range of morphometric cartilage parameters, are helpful for the validation of biochemical biomarkers. In particular, magnetic resonance imaging (MRI) seems more than promising because three-dimensional scan morphometric analysis of cartilage is possible with this method. Both semi-automatic methods for cartilage quantification [14,15] and fully automatic computer-based methods for quantification of a range of morphometric parameters, including cartilage thickness and volume [16–19], have been reported. For instance, the fully automatic method enabled evaluation of the diagnostic (ability to distinguish a group with radiographic OA) and prognostic (prediction of the longitudinal progression in cartilage volume over 21 months) properties of the urinary C-telopeptide of type II collagen (CTX-II) biomarker. Using MRI, several candidate serum and urine biomarkers in patients with knee OA could be qualified for their ability to identify subgroups in which the disease progressed at different rates [20]. Finally, MRI was also used to assess the relevance of potential candidate biomarkers as predictors of the progression of cartilage structural

changes in response to drug treatment in patients with knee OA [21].

Collagen specific neoepitopes as OA biomarkers

The most abundant collagen in articular cartilage is fibrillar type 2 (Coll-2), a triple helix composed of three identical Coll-2 α1 chains. Coll-2 fibers form a tridimensional scaffold responsible for cartilage tensile strength. Other collagens, such as types 3, 9 and 11, stabilize the Coll-2 fibers. Coll-2 molecules are synthesized as propeptides with N- and C-terminal regions that are cleaved extracellularly before final assembly into fibrils. The C- and N-terminal fragments are viewed as potential markers of chondrocyte synthetic activity. The N-terminal peptide (PIINP) also exists in a second form (PIIANP), derived from coll-2 α1 chains that contain a cysteine-rich domain and that are typically expressed by embryonic as well as dedifferentiated, pathological chondrocytes. Immunoassays were set up and validated for measurement of these peptides in biological fluids (Fig. 3). Serum levels of PIIANP were initially shown to be lower in patients with OA than in matched controls [22] and to correlate inversely with cartilage loss at 21 months, as determined by MRI [20] or radiography [23]. At odds with these findings, a five-year longitudinal study involving 135 patients with OA showed instead that increasing concentrations of PIIANP were predictive of OA progression in the knee [24]. Owing to these conflicting results, more studies are needed to assess the value and meaning of this peptide as an OA biomarker.

Native, fibrillar Coll-2 is degraded by MMP-1, -8, -13 and -14, producing three-quarter- and a quarter-length fragments. Denatured, partially degraded Coll-2 is further degraded by gelatinases, namely MMP-2 and MMP-9 and stromelysin (MMP-3). The concerted action of MMPs produces, among others, a C-terminal peptide from the quarter fragment, named CTX-II (Fig. 3). This is probably the most widely characterized collagen fragment used as a urinary marker of cartilage degradation. CTX-II levels were first found to correlate with cartilage loss in animal models of OA. In agreement with these experimental data, clinical studies showed increased CTX-II levels in patients with OA compared with controls [12,24]. CTX-II seems to hold value not only as a diagnostic, but also a prognostic marker. High CTX-II levels were found to be predictive of OA progression assessed initially by X-rays, MRI and clinical assessment [12,24–26]. In other studies, the power of CTX-II as a marker of OA could be increased by combining it with PIIANP to generate a collagen index that takes into account both synthesis and degradation [23]. Nevertheless, some uncertainty still exists about what this peptide reflects, based on the fact that CTX-II is also derived, in part, from calcified cartilage at the interface with bone [12]. At this location, CTX-II would be produced not only by chondrocyte, but also by osteoclast activity.

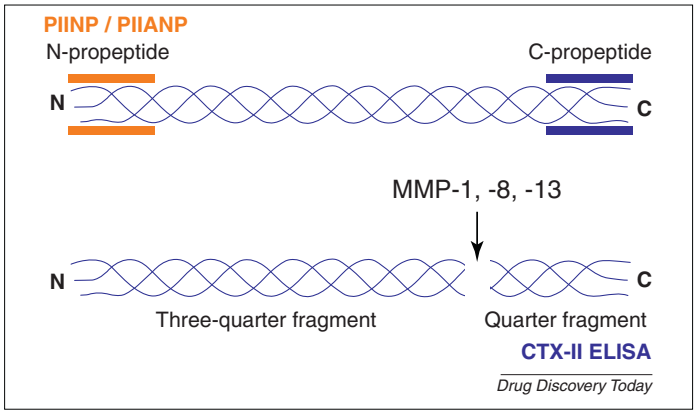

FIGURE 3

Location of the PIINP/PIIANP and CTX-II epitopes in Coll-2.

Aggrecan-specific neoepitopes as OA biomarkers

The main proteoglycan of cartilage is aggrecan, which comprises a large core protein containing three globular domains, G1 to G3. The linear region between G2 and G3 is highly substituted with hydrophilic glycosaminoglycan (GAG) chains of chondroitin sulfate (CS) and keratan sulfate (KS) polymers (Fig. 4). Aggrecan binds, by its G1 domain, to the polymer hyaluronic acid (HA), forming huge macromolecular complexes (up to 300 aggrecan/HA) that are trapped inside the collagen fibrous network and are responsible for the hydrophilic nature of cartilage, and hence its gliding surface, resilience and resistance against compression.

Aggrecan can be degraded by all of the above-mentioned MMPs, which typically cleave the core protein within the G1–G2 interglobular domain at residues VDIPEN$_{341}$–F$_{342}$FGV. Aggrecan is also cleaved by proteases of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin domain) family, namely ADAMTS-4 and -5, (aggrecanases-1 and -2, respectively). These aggrecanases cleave the core protein at multiple sites, first in the G2–G3 CS-rich domain, and then at the signature NITEGE$_{373}$–A$_{374}$RGS position in the G1–G2 interglobular domain (Fig. 4). It is generally agreed that, in early stages of OA, aggrecan is cleaved mainly by aggrecanases, which are joined by MMPs at a later stage, when most of the proteoglycan has already been degraded. Immunoassays are available for fragments generated by both classes of protease. Aggrecanase-generated fragments carrying the neo N-terminal ending A$_{374}$RGS leak out of cartilage and their level in SF, serum and urine is meant to reflect the intensity and stage of cartilage degradation. An ELISA for ARGS was developed and validated for measurement of the neoepitope in SF [27]. The concentration of ARGS in the SF of patients with OA patients was significantly higher than in healthy controls, despite a high scattering of concentration values among patients with OA. Better separation between the normal and pathologic groups was obtained by expressing the neoepitope level as the percentage of total aggrecan present in SF. The relatively low affinity of antiARGS antibodies has long limited the use of ELISA to SF and cartilage culture media. Optimization of an existing antibody by 'directed evolution' recently made an assay possible that can measure the ARGS neoepitope in biological fluids that are more accessible than SF. Preliminary data from 14 patients with moderate OA showed a significant increase in the number of ARGS fragments in serum, but not urine, compared with a control group of the same size [28]. Hopefully, by using this new assay, it will be possible to verify that urinary and/or serum ARGS fragments can be used as markers of catabolic activity in OA, possibly in conjunction with other proteolytic fragments reflecting the activity of MMPs either on aggrecan or collagen. An ELISA is available to measure MMP-generated fragments carrying the neo N-terminal epitope FFGV [29]. By using this assay, it was found that this MMP product increased in serum

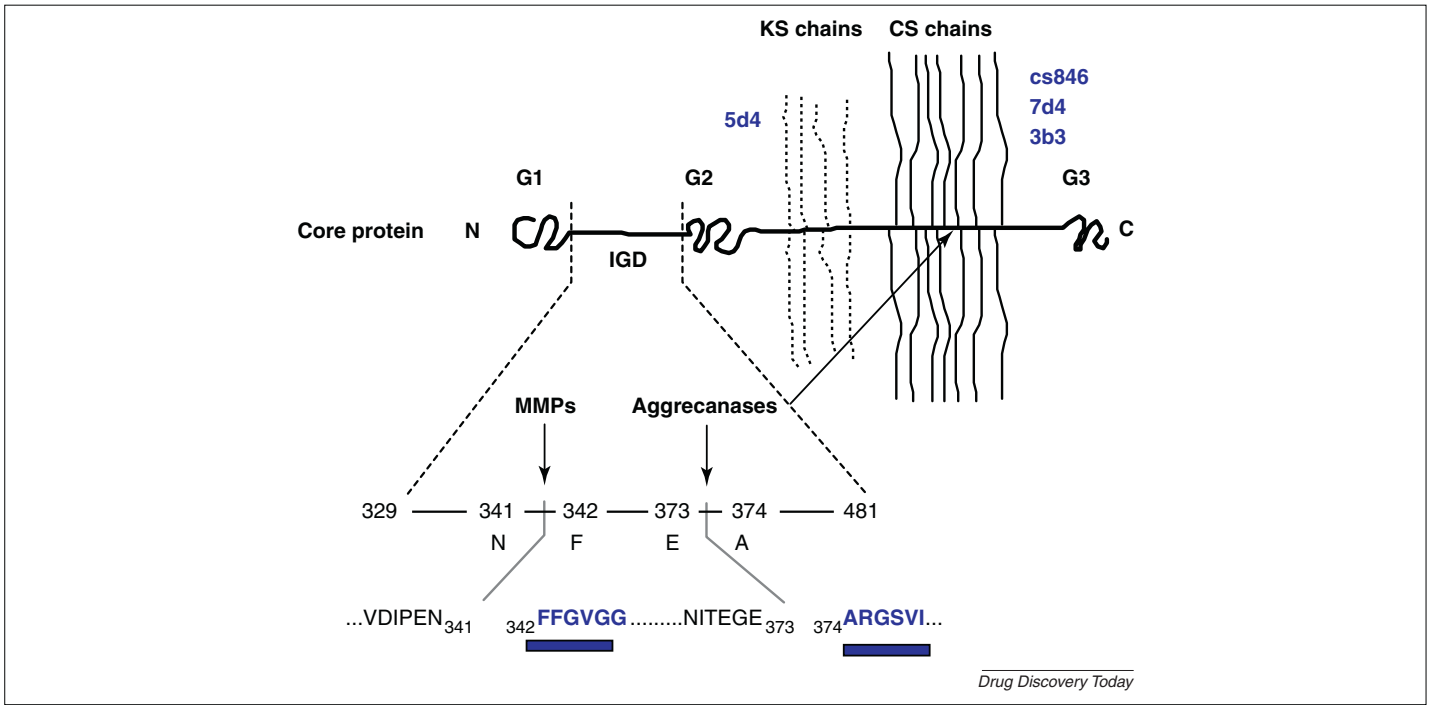

FIGURE 4

Location of epitopes and neoepitopes generated within aggrecan during OA. *Abbreviations*: CS chains, chondroitin sulfate chains; G1, G2, G3, globular domains of aggrecan; IGD, interglobular domain; KS chains, keratan sulfate chains.

of patients with rheumatoid arthritis, compared with healthy controls [29]. However, no data are so far available on the levels of these fragments in patients with OA. A different approach, which does not distinguish between aggrecanase and MMP activity, but has the advantage of reflecting total shedding of aggrecan, is based on antibodies that recognize all fragments carrying G1 and/or G2 globular domains [29]. Similarly to what is seen with the FFGV assay, the only available data concern patients with rheumatoid arthritis, in whom G1/G2 fragments were significantly decreased [29,30].

Non-neoepitopes biomarkers of cartilage remodeling in OA

Although a large number of non-collagenous non-proteoglycans proteins of cartilage have been investigated as possible biochemical markers of OA, none have been consensually agreed as a standard. The most promising candidate, cartilage oligomeric matrix protein (COMP), also known as thrombospondin 5, consists of five identical subunits that bind collagens in the ECM of cartilage. COMP has been proposed as a diagnostic and therapeutic indicator of OA [31]. In a randomly selected cohort of participants with or without radiographically diagnosed knee OA, average serum COMP levels were found to be significantly higher in the OA group and increased with either the severity of the disease or the number of the affected joints [32], as also found in hip OA [33]. Higher baseline levels of serum COMP correlated with more rapid progression of OA, supporting the possible role of COMP as a prognostic factor or early biomarker of OA [34]. However, the actual use of COMP as an OA biomarker still requires some clarification: COMP levels also reflected synovitis [35], which is not an exclusive hallmark of OA, as well as rheumatoid arthritis [36], and large interindividual variabilities were observed in all the above studies. Therefore, the use of COMP as a prognostic, diagnostic or therapeutic marker of OA in individuals rather than in a population group remains uncertain. Interestingly, a peptide fragment of COMP generated by the action of MMP-12 in human articular cartilage might be of value if used as a biomarker in combination with native COMP [37].

Specific structures within CS and KS chains of cartilage proteoglycans have also been proposed as possible biomarkers generated during cartilage remodeling in OA (Fig. 4) (reviewed in [38]). An antibody raised against the CS846 antigen present in the fetal form of aggrecan enabled the detection of this re-expressed CS form in OA cartilage and serum. The 7D4 epitope, consisting of one 6-sulfated and one non-sulfated disaccharide, and the 3B3 epitope, consisting of native CS chains with non-reduced residues of GlcAβ1 and 3GalNac-6-sulfate, were also increased in OA cartilage and in OA biological fluids, probably as the result of an attempt at matrix repair by chondrocytes. The 5D4 epitope, consisting of sequences of *N*-acetyl lactosamine disaccharides of KS proteoglycans, with a minimal epitope requirement of a pentasulfated hexasaccharide, was also proposed as a biochemical marker in OA. Although all these specific GAG motifs were found to be expressed preferentially in OA rather than in healthy cartilage, their level of detection was higher in SF than in serum, and they were also sometimes described as biomarkers of rheumatoid arthritis, possibly limiting their use as specific and easily checked OA biomarkers.

Putting pieces of the puzzle together: the osteoarthritis initiative

Until now, no OA-related biomarker has ever been stringently validated to quantify the total body burden of the disease. This is

essentially because of the current gold standard for diagnosing OA (i.e. plain radiograph), the inaccuracies associated with which still do not enable the qualification of a biomarker for structural endpoints of OA. To address this challenge, the largest public–private partnership, the osteoarthritis initiative (OAI), was launched in 2002 by the National Institutes of Health (NIH) and the Foundation for the National Institutes of Health (FNIH); http://oai.epi-ucsf.org/datarelease/ [39]. The primary objective of this multicentre, longitudinal, prospective observational study of knee OA is to develop an archive of biological specimens that will be available to investigators for the testing and validation of biochemical markers in OA. For the first time, the longitudinal nature of this study (4 years), based on a large cohort of participants (~1300 with established OA of the knee and ~3500 with significant risk factors for the development of knee OA), will allow correlations of changes within a person over time between different elements of disease, including measures of structural changes, assessed by X-rays (osteophytes and JSN) and MRI (notably cartilage volume and lesions scores), and disability and pain. The OAI will support analyses that researchers might want to perform to evaluate putative biomarkers and to assess their potential for surrogacy and it is designed to have adequate precision for estimating the joint relationship between proposed biomarkers and desired endpoints. At the very least, investigators will be able to identify promising and relevant biomarkers for use in the early development of treatments and that can be tested and/or validated in trials as surrogates for treatment effects.

Concluding remarks

The interest of the scientific community in the identification of OA biomarkers has been increasing over the past few years, spurred on by worldwide initiatives, including the launch of the BJD in 2000 and the OAI in 2002. There is no doubt that such common efforts will lead to success in the next few years. Given constraints owing, in part, to the heterogeneity of OA, markers in combination will be the only possible approach, not only practically for sensitivity concerns, but also for increasing confidence in the specific detection of OA and possibly of subgroups of patients with OA. Recent work demonstrated that biochemical marker combinations are more appropriate than are individual biochemical markers for reflecting structural damage in patients with OA [13,20,21,34,40,41]. In this context, it also seems likely that bone-derived biomarkers are helpful in combination with proteins or protein degradation products coming from cartilage itself. Interestingly, changes in bone metabolism occurring during the progression of OA and monitored by biochemical markers might also provide substantial information on the efficacy of treatments aimed at targeting bone and cartilage simultaneously [42–44]. We also emphasize that 'omics' approaches, including proteomics [45], metabolomics [46], lipidomics [47] and degradomics [37], have been underexploited until now, although it seems obvious that they could open new avenues of discovery and implement the important, but still incompletely validated, arsenal of candidate biochemical markers of OA.

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