ELSEVIER

Proteomics: addressing the challenges of osteoarthritis

Frédéric De Ceuninck[1] and Francis Berenbaum[2]

[1] Servier Research Institute, Department of Rheumatology, 11 rue des Moulineaux, 92150 Suresnes, France
[2] UR 4, UPMC Paris VI University, AP-HP, Saint-Antoine Hospital, 184 rue du faubourg Saint-Antoine, 75012 Paris, France

Osteoarthritis (OA) is the most frequent rheumatic disease and is the leading cause of disability in developed countries. Its poorly understood pathophysiology limits the discovery of targets for pharmacological intervention and there are few effective medical treatments beyond pain control and surgery. Proteomic technologies may help identifying new targets of OA as well as diagnostic or therapeutic biomarkers, which has stimulated interest in this field. In this review, we discuss the most recent findings arising from the use of proteomics for the identification of OA biomarkers in synovial fluid and serum, and for the discovery of possible therapeutic targets in cartilage or by using chondrocyte culture systems.

Introduction

Osteoarthritis (OA) is the leading cause of disability in developed countries. In addition to hereditary components, ageing and obesity are the two main risk factors of OA explaining why its incidence is dramatically increasing [1]. More than 25 million Americans were affected in 2005 with eighty percent being over 50 years old [2]. The sex ratio of OA is two females for one male. Pain, loss of function and stiffness are the main clinical symptoms of the disease **(**Figure 1**),** knee, hip, hand and spine being the most frequently affected sites [3]. OA mostly affects articular cartilage, a soft connective tissue that covers and protects the end of bones in all synovial joints. Although only a few millimeters thick, articular cartilage has an incredibly low coefficient of friction which, coupled with its ability to bear very large compressive loads, ensures joint mobility. OA is characterized by a progressive loss of articular cartilage and in advanced cases, its total loss leads to friction between adjacent bones and limitation of joint mobility. In OA, cartilage degeneration is generally associated with alterations in the subchondral bone area, inflammation and neovascularization processes. The method generally used for diagnosis of OA is X-ray, showing joint space narrowing, osteophyte formation and subchondral bone sclerosis **(**Figure 1**)**. Pharmacological treatments are essentially symptomatic and include oral (systemic) or topical remedies, intraarticular therapies, and dietary supplements, all of which may only reduce pain. Unfortunately, no drug has yet demonstrated any disease-modifying activity, which explains why the number of surgical interventions for total joint prosthesis, the only real 'cure' for OA, is increasing in parallel with the growing incidence of the disease. Although several drugs acting in the subchondral bone area and/or targeting various cytokines and growth factors have been moved forward into clinical development [4], focusing efforts on the identification of new drug targets would undoubtedly lead to the identification of treatments addressing the causes of OA. However, in order to reliably monitor OA progression and assess treatment efficacy, surrogate markers are desperately needed [5,6]. Proteomic technologies are particularly well suited for the identification of proteins involved in the etiology and progression of most pathologies, as well as for the identification of diagnostic or therapeutic biomarkers.

Clinical heterogeneity of OA: a complication for proteomic analyses

The application of proteomic technologies in OA is not straightforward due to the inherent complexity of this disease. The clinical heterogeneity is a hallmark of OA. The progression of OA is slow and periodic, with intermittent episodes of inflammatory flares and remission periods, so the contribution of proteomics to the

Corresponding author: De Ceuninck, F. (frederic.deceuninck@fr.netgrs.com)

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2009.05.006

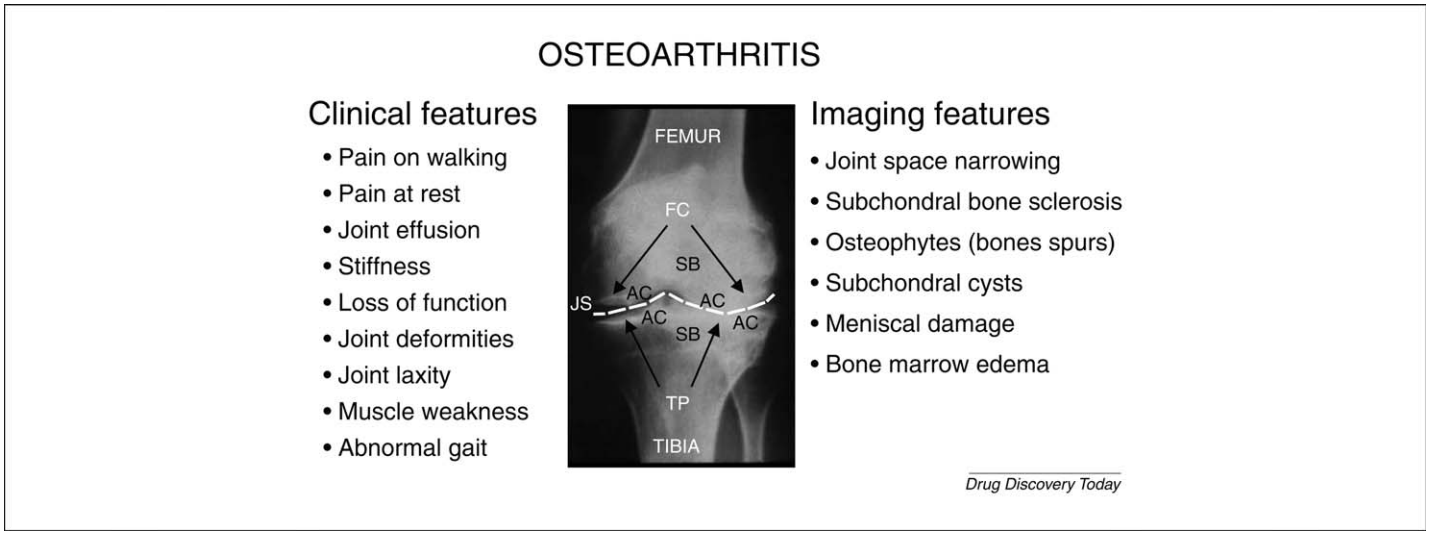

FIGURE 1

Clinical and Imaging features of osteoarthritis. The central picture illustrates an X-ray photograph of the knee joint, one of the sites most frequently affected by osteoarthritis. Abbreviations: FC, femoral condyles; TP, tibial plateaus; SB, subchondral bone; AC, articular cartilage; JS, joint space.

successful identification of potential drug targets or biomarkers during the course of the disease requires thorough clinical assessment. Although serum and urine biomarkers for diagnosis and prognosis have been proposed recently [5,6], none of them can be used in clinical practice because of large interindividual variations. Furthermore, potential biomarkers released into the synovial fluid during the course of cartilage remodeling are highly diluted in plasma and urine, and their identification requires highly sensitive and reliable techniques. The identification of drug targets in early OA is problematic as OA is rarely detected before the appearance of pain symptoms, which occurs at a more advanced stage of the disease. In addition, it is difficult to obtain healthy and OA human cartilage for comparative studies and it should be noted that OA cartilage is heterogeneous, with intact and moderately to severely damaged cartilage zones coexisting in a single affected joint. In healthy cartilage, the density of functional cells (chondrocytes) is low, and it is even lower in OA cartilage. Nevertheless, recent progress made in the development of sensitive and reliable proteomics tools is promising to bypass these difficulties. A list of proteomics techniques that have been used for the identification of drug targets and biomarkers of OA is summarized in Table 1. In addition, an overview of the strategies that have been or could be used for proteomic intervention in OA is illustrated in Figure 2 and detailed below.

Delving into the core of OA: cartilage proteomics

The nature of cartilage composition and its implication for proteomic analyses

Cartilage proteomics primary aims at the identification of new drug targets for the treatment of OA. Cartilage predominantly consists of water, with a high concentration of anionic macromolecules, including hyaluronan and the highly sulfated proteoglycan aggrecan, and collagens, all of which build the extracellular matrix. Only a few chondrocytes are scattered within the extracellular matrix. In normal conditions, these highly specialized cells synthesize the cartilage components and their main role is to maintain a balance between synthesis and degradation of extracellular matrix. During OA, this balance is not maintained and dysregulation of chondrocyte metabolism leads to progressive degradation of the cartilage matrix. It is commonly accepted that metalloproteinases and aggrecanases induced by catabolic cytokines such as interleukin-1 (IL-1) and tumor necrosis factor α (TNFα) favor the degradation of extracellular matrix proteins. Much attention has been focused on cartilage in order to identify some proteins playing a role in the etiology of OA. However, the molecular composition of cartilage presents huge technical problems. To analyze the cartilage proteome, it is necessary to discard collagens and proteoglycans that interfere with the first analytical steps, while maintaining the integrity of the small amount of important proteins. One way this was successfully achieved was by prior aggregation of proteoglycans with the cationic detergent cetylpyridinium chloride, which then allowed better analysis of proteins extracted from pathologic human cartilage [7]. Similar qualitative electrophoretic profiles of about 600 proteins were obtained with cartilage coming from patients with early OA, rheumatoid arthritis, psoriatic arthritis or chondrosarcoma, demonstrating that it was possible to depict a global 'cartilage signature'. However, the large amount of albumin remaining in protein extracts meant that informative proteins were probably underrepresented, pointing out the necessity to develop complementary or alternative methods in order to detect low abundance proteins. In a recent study, cartilage proteins of OA patients were analyzed after digestion of the cartilage tissue with a collagenase cocktail [8]. Over 100 different proteins could be identified from nearly 700 peptide sequences. However, a filtration step used to remove collagenase in this procedure had probably led to the loss of some important cartilage proteins. Recently, two groups independently reported improved procedures for the analysis of cartilage proteins starting from murine cartilage samples [9,10]. Pecora *et al.* used a passive rehydration loading approach that reduced the entry of proteoglycans and collagens into immobilized pH gradient (IPG) strips. The advantage of this method was that no sample pretreatment was required, thus minimizing the possibility of selective protein loss [9]. In order to improve the

TABLE 1

Analytical platforms used for proteomic studies of OA

Materials	Analytical platform	References
Cartilage	2-DE/MS	Vincourt *et al.* [7]
		Pecora *et al.* [9]
		Wilson and Bateman [10]
		Guo *et al.* [12]
	1-D-SDS-PAGE/LC–MS	Garcia *et al.* [8]
		Wu *et al.* [11]
Culture medium/buffer of cartilage explants	2-D DIGE/MS	Wilson *et al.* [13]
	2-DE/LC–MS	Hermansson *et al.* [17]
	2-DE/MS	De Ceuninck *et al.* [18]
	Off-Gel electrophoresis/MS	De Ceuninck *et al.* [18]
	Antibody microarrays	De Ceuninck *et al.* [18]
	LC–MS	Zhen *et al.* [19]
Chondrocytes	2-DE/MS	Ruiz-Romero *et al.* [21]
		Ruiz-Romero *et al.* [22]
		Lambrecht *et al.* [30]
	2-D DIGE/MS	Rollin *et al.* [23]
Culture medium of chondrocytes	Antibody microarrays	De Ceuninck *et al.* [24]
	2-DE/MS	Catterall *et al.* [25]
Chondrocyte mitochondria	2-DE/MS	Ruiz-Romero *et al.* [27]
	2-D DIGE/MS	Ruiz-Romero *et al.* [28]
Synovial fluid	2-DE/MS	Sinz *et al.* [14]
	2-DE	Yamagiwa *et al.* [31]
	1-D-SDS-PAGE/LC–MS	Gobezie *et al.* [32]
	LC–MS	Kamphorst *et al.* [34]
		Steinbeck *et al.* [35]
	LC/2-DE	Jmeian and El Rassi [36]
Serum	Antibody microarrays	Ling *et al.* [37]
	2-DE/MS	Xiang *et al.* [38]
		Xiang *et al.* [39]

2-DE: two-dimensional electrophoresis, MS: mass spectrometry, LC–MS: liquid chromatography–mass spectrometry, 2D-DIGE: two-dimensional difference gel electrophoresis, OA: osteoarthritis.

extraction of proteins from cartilage, Wilson and Bateman used a sequential fractionation procedure with a NaCl buffer that extracted mostly intracellular proteins and with a guanidinium chloride buffer that disrupted tightly interacting matrix components [10]. These two methods were claimed to be efficient and reproducible and thus suited to prepare samples for the comparison of normal and pathological proteomes.

Differential proteomics comparing normal with OA cartilage

As if to confirm the difficulty to establish reliable and reproducible proteome maps of cartilage tissue, only two studies have so far been published comparing normal and OA cartilage proteomes [11,12] **(**Table 2**)**. In the first study, more than 800 proteins were identified, and 59 proteins were found to be increased in OA cartilage compared with normal cartilage [11]. Among these, proteins known to be involved in the degradation of the extracellular matrix of cartilage such as the serine protease Htra1 and matrix metalloproteinase-2 were identified, as well as anti-catabolic factors such as TIMP-2 and cystatin c, demonstrating that certain mechanisms to counteract cartilage loss were still functioning in OA cartilage. Other proteins involved in lipid metabolism, immune responses and signal transduction were also identified as being dysregulated in OA cartilage. Guo *et al.* identified more than 1400 protein spots in OA cartilage [12]. Eight spots were found to be increased and eight spots were found to be decreased in OA cartilage compared to normal cartilage. Five of these proteins were known to be involved in the regulation of glycolysis and energy production, and the authors suggested that a resulting decrease of the ATP/ADP ratio could be responsible for the decreased ability of chondrocytes to combat efficiently degradation of the extracellular matrix.

Cartilage explant models

Cartilage explants incubated in a culture medium may be treated so as to reflect the behavior of native cartilage in a (patho)physiological environment. On the basis of this assumption, *in vitro* models of cartilage explants have been used to understand protein changes occurring in OA cartilage and to identify potential drug targets. Moreover, the identification of proteins released into the culture medium may give clues about potential biomarkers released into the synovial fluid during the course of cartilage degradation in OA. The catabolic cytokines IL-1 and TNFα were used to induce pathological changes in cartilage explants and to identify protein degradation products released into the culture medium. Using this approach, however, required certain precautions such as the use of fresh cartilage biopsies to maintain chondrocyte viability, and removal of fetal calf serum in the medium. Mouse cartilage explants stimulated with IL-1α released high levels of calgranulin B and cyclophilin B [13], two proteins

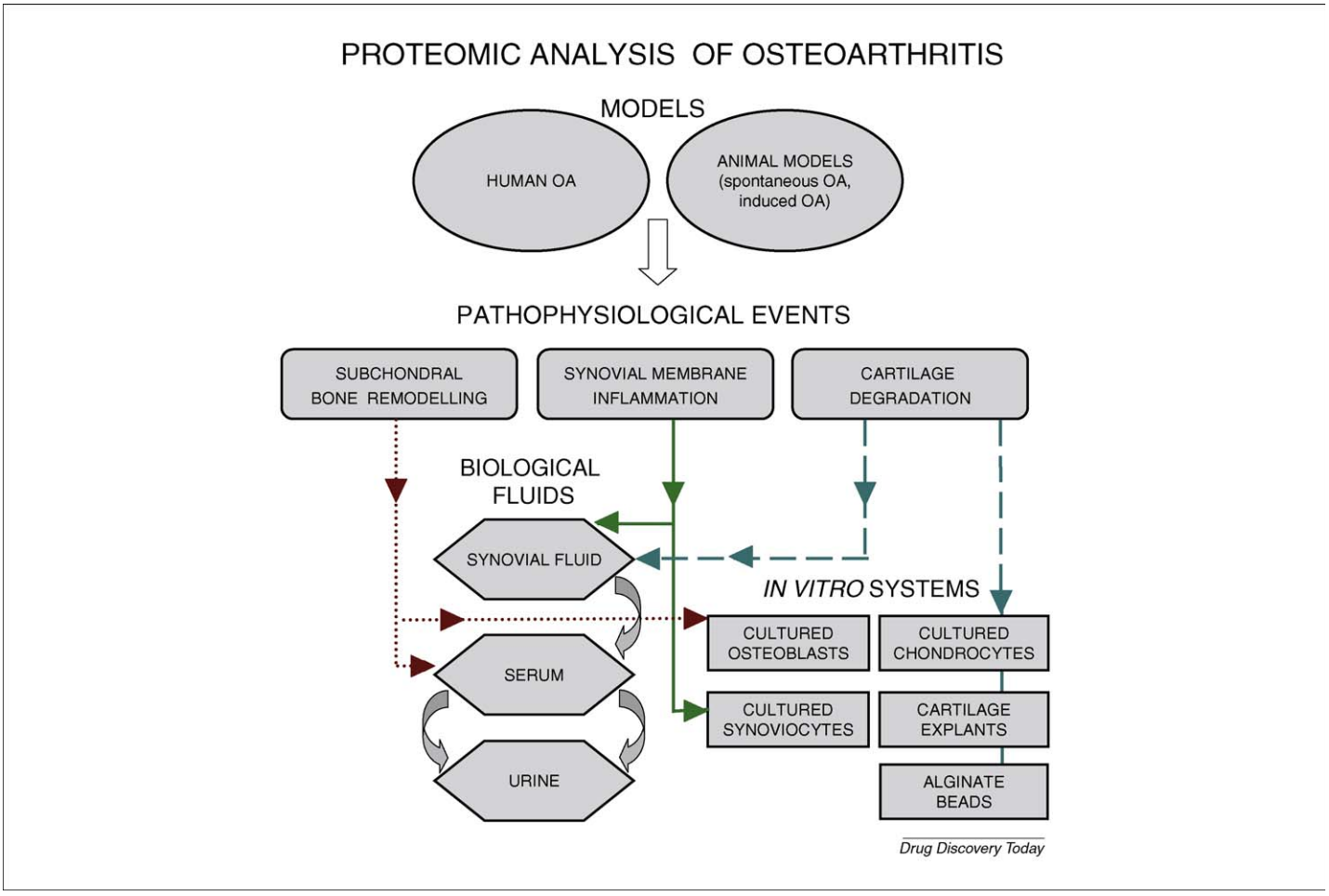

FIGURE 2

Proteomic intervention in osteoarthritis. Samples from osteoarthritic patients or animal models of osteoarthritis (OA) can be used for the identification of disease targets or biomarkers of OA. Pathophysiological events arising in the subchondral bone, synovial membrane or cartilage can induce the release of specific proteins or protein fragments into biological fluids. Proteomic technologies can help in the identification of biomarkers in synovial fluid, serum or urine. Moreover, *in vitro* systems of cartilage degradation, synovial inflammation or subchondral bone remodeling can be analyzed by proteomics to better the understanding of the underlying mechanisms of OA and identify drug targets.

previously described as being more closely associated to rheumatoid arthritis than OA [14,15]. Additionally, YKL-40, a protein known to act as a growth factor for cartilage [16], matrix metalloproteinase-3, an enzyme involved in the degradation of the extracellular matrix, and proteins newly identified in cartilage, haptoglobin, and neutrophil gelatinase-associated lipocalin (NGAL), were also increased in the medium of cartilage stimulated with IL-1α. Using different techniques, two independent groups identified similar proteins released by human OA cartilage explants, such as the pigment epithelium derived factor (PEDF), osteoglycin/mimecan, and the serum amyloid-P component [17,18], in addition to proteins originating from serum, synovial fluid, or the subchondral bone, thereby demonstrating that cartilage is under the influence of proteins from its surrounding tissues/fluids. The presence of serum proteins within OA cartilage also underlined that neovascularization is a hallmark of OA progression. Antibody microarray analysis of the culture medium of OA cartilage explants also demonstrated high levels of eleven proteins previously known to be involved in inflammatory, angiogenic, degradative and reparative processes, including tissue inhibitor of metalloproteinases-1 and -2 (TIMP-1, TIMP-2), vascular endothelial growth factor (VEGF), monocyte chemoattractant protein-1 (MCP-1), angiogenin, thrombopoietin and osteoprotegerin [18]. In order to identify new possible markers of cartilage degradation, Zhen *et al.* examined proteolyzed peptide products induced by the action of activated metalloproteinases on human cartilage explants [19]. A wide variety of peptides originating from type-I, -II and -III collagens, aggrecan, small leucine rich proteoglycans, cartilage oligomeric matrix protein (COMP) and cartilage intermediate-layer protein (CILP) were identified. These findings provided insights into the large number of substrates susceptible to specific metalloprotease proteolysis in diseased cartilage, and raised the possibility that these peptides may represent potential markers of OA.

Cell culture models

The use of primary chondrocytes in culture is also relevant for the proteomic identification of OA targets **(**Table 2**)**. Because of the paucity of chondrocytes in cartilage, cells must first be grown in culture before analysis. The main advantage of using chondrocytes rather than cartilage is to reduce the complexity of the biochemical procedures used to extract proteins for further analysis. The

TABLE 2

Selected potential targets/biomarkers of osteoarthritis identified by proteomics

Protein	Materials	Function	Increased or decreased in OA	References
Htra 1, MMP-2	Cartilage	Protease	↑	Wu *et al.* [11]
Timp-2, cystatin-c	Cartilage	Protease inhibition	↑	Wu *et al.* [11]
Alcohol dehydrogenase, pyruvate kinase, α enolase,	Cartilage	Energy homeostasis	↑	Guo *et al.* [12]
Adenylate kinase, flavin reductase	Cartilage	Energy homeostasis	↓	Guo *et al.* [12]
Thioredoxin dependent peroxide reductase, superoxide dismutase	Cartilage	Redox	↓	Guo *et al.* [12]
HSP90β, GRP78, GRP94	Chondrocytes (monolayers)	Stress response	↑	Ruiz-Romero *et al.* [22]
G3PDH, enolase, fructose biphosphate aldolase	Chondrocytes (monolayers)	Energy homeostasis	↓	Ruiz-Romero *et al.* [22]
TRAP-1	Chondrocyte mitochondria	Counteracts oxidative stress	↑	Ruiz-Romero *et al.* [27]
Superoxide dismutase 2	Chondrocyte mitochondria	Antioxidant properties	↓	Ruiz-Romero *et al.* [27]
Vimentin cleavage products	Chondrocytes in alginate	Cytoskeleton organization	↑	Lambrecht *et al.* [30]
Apolipoprotein A-IV	Serum	Lipid metabolism	↑	Jmeian and El Rassi [36]
Apolipoprotein A-I	Serum	Lipid metabolism	↓	Jmeian and El Rassi [36]
VE-cadherin	Serum	Cell adhesion	↑	Ling *et al.* [37]
BLC, MIP-1α, IL-2	Serum	Immune response, inflammation	↑	Ling *et al.* [37]
MMP-7	Serum	Protease	↑	Ling *et al.* [37]
NT-4	Serum	Growth factor	↑	Ling *et al.* [37]
Triosephosphate isomerase and fibulin-4 autoantibodies	Serum	(in these studies), autoantigens	↑	Xiang *et al.* [38,39]

MMP-2: matrix metalloproteinase 2, NT-4: neurotrophin-4, MIP-1α: macrophage inflammatory protein-α.

difficulty with this approach is that chondrocytes dedifferentiate into fibroblast-like cells in culture and it is necessary to use cells at high density and avoid passages [20]. After a pilot study to establish a 2-DE map of the human normal chondrocyte proteome [21], comparative proteomic analyses allowed the identification of 28 proteins which were differentially expressed in OA chondrocytes compared to normal cells [22]. Three stress response proteins (heat shock protein 90β, Glucose-regulated 78 kDa protein and Glucose-regulated 94 kDa protein) were found to be increased in OA cells, whereas three proteins involved in energy production (Glyceraldehyde 3 phosphate dehydrogenase, enolase and fructose biphosphate aldolase) were decreased. A similar result was obtained in OA cartilage [12], suggesting that the decrease of ATP/ADP ratio could be a key event in OA. A comparative analysis between cultured chondrocytes coming from healthy donors or patients with end-stage OA by two-dimensional difference gel electrophoresis (DIGE) identified an altered expression of 27 proteins involved in cytoskeleton organization, apoptosis, glycolysis, protein folding and protein degradation [23]. The use of antibody microarrays to analyze the secretome of human OA chondrocytes in culture revealed several unexpected proteins, including epidermal growth factor (EGF), thrombopoietin, glial cell-line derived neurotrophic factor (GDNF), neurotrophins-3 and -4 (NT-3, NT-4), as well as a wide variety of chemokines [24]. These findings suggested that the intrinsic proinflammatory equipment of OA chondrocytes may play an important part in the pathogenesis and/or progression of OA. Using 2-DE, OA chondrocytes stimulated by the catabolic cytokines IL-1 or oncostatin M were found to release small peptide fragments of matrix metalloproteinases-1, -3, YKL-40, and cyclophilin A [25]. There is also increasing evidence that mitochondrial dysfunction in OA chondrocytes mediates pathways involved in cartilage degradation, including alterations in respiratory chain complexes, increased oxidative stress and apoptosis [26]. Following the establishment of a 2-DE reference map of the mitochondrial proteome of normal articular chondrocytes [27], DIGE was used to compare normal and OA human mitochondrial proteomes [28]. TRAP-1, a chaperone with reactive oxygen species antagonist activity, was found to be increased in OA chondrocytes. This can be interpreted as a possible compensational mechanism to the increased oxidative stress environment. At the same time, superoxide dismutase 2 (SOD2), a protein with antioxidant properties was decreased in OA chondrocytes. In order to avoid the dedifferentiation of chondrocytes cultured in monolayers, the culture of chondrocytes in collagen sponges or in alginate beads has also been used for proteomic investigations [29,30]. Increased vimentin cleavage products were identified in human OA chondrocytes compared to normal chondrocytes cultured in alginate beads, revealing a possible disorganization of the OA chondrocyte cytoskeleton [30].

Proteomic analysis of synovial fluid in OA

It is obvious that progress in monitoring the efficacy of OA treatments would be improved with the identification of biomarkers. Accordingly, proteomic techniques have been used to search new OA biomarker candidates in the synovial fluid (SF) and serum of OA patients. Because of a higher concentration of cartilage degradation products in the SF compared to serum, most studies looking for OA biomarkers have focused on the SF of the affected joints. While this is a reasonable approach for research, a biomarker from SF would be impractical for large scale clinical diagnosis and monitoring. Moreover, the abundance of the anionic macromolecule hyaluronan in SF complicates biomarker detection. Before proteomic analysis, SF samples need to be subjected to various separation procedures including hyaluronidase treatment to remove

hyaluronan, centrifugation to remove contaminating cells, and affinity chromatography to remove abundant proteins such as albumin and immunoglobulins, which mask the detection of lower abundance proteins. In a study aiming at the identification of biomarkers in the SF of OA patients by 2-DE, Yamagiwa *et al.* demonstrated a high level of interindividual heterogeneity with differences of more than 100-fold for certain proteins [31]. In a larger cohort of patients with early or late OA and healthy individuals, 135 high-abundance SF proteins were identified in all three groups. Eighteen proteins were found to be differentially expressed between healthy individuals and OA patients, but no significance between patients with early or late OA could be identified [32]. In two other studies aiming at comparing the proteomes of SF obtained from patients with OA and various inflammatory joint disorders, some proteins were found to be more related to inflammatory arthritis than OA [14,33]. A gel-free technique was claimed to be robust, on the basis of the successful profiling of peptides in the SF from an OA patient and a healthy control [34]. In parallel with an increase of myeloperoxidase, an enzyme responsible for the production of highly reactive hypochlorous acid, elevated levels of chlorinated peptides were identified by LC–MS in the SF of patients with early or mild OA, but were barely detectable in healthy controls or patients with late OA [35].

Proteomic analysis of serum in OA

Because of easier access, serum has been investigated as a possible source for specific OA biomarkers **(**Table 2**)**. However, as mentioned above, biomarkers derived from a slow and focal cartilage degradation process are expected to be highly diluted in serum and therefore require highly sensitive and reliable detection techniques. In order to deplete high-abundance proteins from sera taken from control or OA patients, Jmeian and El Rassi developed an integrated microcolumn-based fluidic platform followed by 2-DE analysis [36]. 2-DE maps indicated that apolipoprotein A-IV was increased and apolipoprotein A–I decreased in the serum of OA compared to healthy donors. Furthermore, it was demonstrated that supplementing the diet of OA patients with soy protein decreased the levels of serum hemopexin, whereas kininogen, the vitamin D-binding protein, and transthyretin were increased after treatment. Unfortunately, the correlation between protein changes and clinical efficacy of this treatment was not studied, casting doubt on the possible use of these proteins as biomarkers of OA. Recently, a microarray platform was used to identify sixteen proteins differentially expressed in the serum of OA patients compared to control individuals [37]. The most noticeable changes were found for VE–cadherin, interleukin-2 (IL-2), macrophage inflammatory protein-1α (MIP-1α), NT-4, vascular adhesion protein-1 (VAP-1), matrix metalloproteinase-7 (MMP-7), and B lymphocyte chemoattractant (BLC), with increases by 20–35% above control individuals. By using 2-DE nitrocellulose maps of chondrocyte-derived proteins incubated with the serum of patients with different arthritic disorders, another study elegantly demonstrated the presence of specific autoantibodies against triosephosphate isomerase and fibulin-4 in the serum of OA patients [38,39]. It was suggested that OA-specific autoantigens may drive chronic synovitis and lead to an increased production of cytokines and proteinases responsible for cartilage degradation [40].

Future research

We emphasize that some emerging areas of investigation deserve further attention. Although excessive remodeling is known to exist in the subchondral bone of OA patients [41], no proteomic study has addressed this question yet. Interestingly, differential proteome analysis of bone marrow mesenchymal stem cells between healthy donors and OA patients suggested that cells coming from OA patients may be activated in response to chemotactic signals sent by the altered subchondral bone, in an attempt to heal damaged tissue [42]. It is also noteworthy that animal models of naturally occurring or induced OA have not been properly investigated yet, despite their obvious advantage in bypassing the heterogeneity of human OA. Using proteomics to analyze such models could help detecting new relevant biomarkers of OA at early stages of the disease, as well as monitoring the efficacy or the protein changes occurring after the administration of new pharmacological treatments. Future fields of investigation should also include the use of phosphoproteomic tools, as the modification of the ATP/ADP ratio in OA cartilage may lead to profound alterations in the activity of an important number of proteins involved in normal chondrocyte functioning. Finally, on the fringe of proteomics, the recent analysis of glycosaminoglycan profiles in cartilage by glycomics, using an amide–hydrophilic interaction chromatography online LC–MS/MS procedure [43], was demonstrated to be promising for the identification of glycosaminoglycans changes occurring in OA cartilage.

Conclusions

OA proteomics is still in its early years. The studies of this past decade brought to the forefront the existence of a multitude of pathophysiological events, including increased cartilage degradation due to an imbalance between catabolic and repair processes, mitochondrial dysfunction, immunological mechanisms, and inflammation. Although OA was initially considered merely a disease of cartilage, proteomic studies have confirmed the importance of tissues surrounding cartilage and biological fluids in the etiology and progression of this disease. However, the diversity of approaches and models, together with the heterogeneity of OA samples has not allowed the identification of consensual drug targets or biomarkers yet. In this respect, the relevance of proteins proposed as OA biomarkers will need to be addressed in longitudinal studies using large cohorts of patients at different stages of the disease. It should also be noted that most studies used 2-DE, a method that is known to detect only 30–50% of the proteome depending on the sample analyzed, and often shows significant gel-to-gel variation. Techniques such as 2D-DIGE, which enables the simultaneous analysis of two samples, may circumvent gel-to-gel variability. It is also known that degradation of cartilage matrix proteins by metalloproteinases in OA generates a large number a peptide fragments, which cannot be detected by 2-DE. As these peptide fragments represent potential OA biomarkers, gel-free techniques that have proven to be useful for such identifications clearly need to be used as more global approaches [19]. Undoubtedly, progress towards these

directions should increase our knowledge of this complex pathology, and help in the identification of druggable targets and of specific clinical biomarkers.

Acknowledgement

The authors would like to thank Dr Brant De Fanti for careful rereading of the manuscript.

References

1 Walker-Bone, K. *et al.* (2000) Medical management of osteoarthritis. *Br. Med. J.* 321, 936–940
2 Lawrence, R.C. *et al.* (2008) Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. *Arthritis Rheum.* 58, 26–35
3 Sellam, J. and Berenbaum, F. (2008) Clinical features of osteoarthritis. In *Kelley's Textbook of Rheumatology* (8th edition) (Firestein, G.S., Budd, R.C., Harris, E.D., McInnes, I.B., Ruddy, S., Sergent, J.S., eds), pp. 1547–1562, Elsevier/Saunders
4 Michon, M. *et al.* (2009) Management of osteoarthritis : pharmacotherapy. In *Targeted Treatment of the Rheumatic Diseases* (1st edition) (Weisman, M.H., Weinblatt, M.D., Louie, J.S. and Van Vollenhoven, R. eds), Saunders Elsevier, ISBN-13: 978-1-4160-9993-2, in press
5 Kraus, V.B. (2005) Biomarkers in osteoarthritis. *Curr. Opin. Rheumatol.* 17, 641–646
6 Rousseau, J.C. and Delmas, P.D. (2007) Biological markers in osteoarthritis. *Nat. Clin. Pract. Rheumatol.* 3, 346–356
7 Vincourt, J.B. *et al.* (2006) Establishment of a reliable method for direct proteome characterization of human articular cartilage. *Mol. Cell. Proteomics* 5, 1984–1995
8 Garcia, B.A. *et al.* (2006) Protein profile of osteoarthritic human articular cartilage using tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* 20, 2999–3006
9 Pecora, F. *et al.* (2007) A quantitative and qualitative method for direct 2-DE analysis of murine cartilage. *Proteomics* 7, 4003–4007
10 Wilson, R. and Bateman, J.F. (2008) A robust method for proteomic characterization of mouse cartilage using solubility-based sequential fractionation and two-dimensional electrophoresis. *Matrix Biol.* 27, 709–712
11 Wu, L. *et al.* (2007) Comparative proteomic characterization of articular cartilage tissue from normal donors and patients with osteoarthritis. *Arthritis Rheum.* 56, 3675–3684
12 Guo, D. *et al.* (2008) Proteomic analysis of human cartilage: identification of differentially expressed proteins in knee osteoarthritis. *Joint Bone Spine* 75, 439–444
13 Wilson, R. *et al.* (2008) Proteomic characterization of mouse cartilage degradation *in vitro*. *Arthritis Rheum.* 58, 3120–3131
14 Sinz, A. *et al.* (2002) Mass spectrometric proteome analysis of synovial fluids and plasmas from patients suffering from rheumatoid arthritis and comparison to reactive arthritis or osteoarthritis. *Electrophoresis* 23, 3445–3456
15 De Ceuninck, F. *et al.* (2003) High binding capacity of cyclophilin B to chondrocyte proteoglycans and its release from the cell surface by matrix metalloproteinases: possible role as a proinflammatory mediator in arthritis. *Arthritis Rheum.* 48, 2197–2206
16 De Ceuninck, F. *et al.* (2001) YKL-40 (cartilage gp-39) induces proliferative events in cultured chondrocytes and synoviocytes and increases glycosaminoglycan synthesis in chondrocytes. *Biochem. Biophys. Res. Commun.* 285, 926–931
17 Hermansson, M. *et al.* (2004) Proteomic analysis of articular cartilage shows increased type II collagen synthesis in osteoarthritis and expression of inhibin βA (activin A), a regulatory molecule for chondrocytes. *J. Biol. Chem.* 279, 43514–43521
18 De Ceuninck, F. *et al.* (2005) Assessment of some tools for the characterization of the human osteoarthritic cartilage proteome. *J. Biomol. Tech.* 16, 256–265
19 Zhen, E.Y. *et al.* (2008) Characterization of metalloprotease cleavage products of human articular cartilage. *Arthritis Rheum.* 58, 2420–2431
20 Thirion, S and Berenbaum, F. (2004) Culture and phenotyping of chondrocytes in primary culture. In *Cartilage and Osteoarthritis Volume 1: Cellular and Molecular tools* (Sabatini M., Pastoureau P. and De Ceuninck F., eds), *Methods Mol. Med.* 100, 1–14, Humana Press
21 Ruiz-Romero, C. *et al.* (2005) Proteomic characterization of human normal articular chondrocytes: a novel tool for the study of osteoarthritis and other rheumatic diseases. *Proteomics* 5, 3048–3059
22 Ruiz-Romero, C. *et al.* (2008) Proteomic characterization of human osteoarthritic chondrocytes reveals protein changes in stress and glycolysis. *Proteomics* 8, 495–507
23 Rollin, R. *et al.* (2008) Differential proteome of articular chondrocytes from patients with osteoarthritis. *J. Proteomics Bioinform.* 1, 267–280
24 De Ceuninck, F. *et al.* (2004) The inflammatory side of human chondrocytes unveiled by antibody microarrays. *Biochem. Biophys. Res. Commun.* 285, 926–931
25 Catterall, J.B. *et al.* (2006) Development of a novel 2D proteomics approach for the identification of proteins secreted by primary chondrocytes after stimulation by IL-1 and oncostatin M. *Rheumatology* 45, 1101–1109
26 Blanco, F. *et al.* (2007) Mitochondria and chondrocytes: role in osteoarthritis. In *Osteoarthritis, Inflammation and Degradation: A Continuum* (Buckwalter, J.A., Lotz, M., Stoltz, J.F., eds), pp. 192–205, IOS Press, Amsterdam
27 Ruiz-Romero, C. *et al.* (2006) Mitochondrial proteomic characterization of human normal articular chondrocytes. *Osteoarthritis Cartilage* 14, 507–518
28 Ruiz-Romero, C. *et al.* (2009) Mitochondrial dysregulation of osteoarthritic human articular chondrocytes analyzed by proteomics. *Mol. Cell. Proteomics* 8, 172–189
29 Freyria, A.M. and Becchi, M. (2004) Changes of chondrocyte metabolism *in vitro*: an approach by proteomic analysis. In *Cartilage and Osteoarthritis Volume 1: Cellular and Molecular Tools* (Sabatini M., Pastoureau P. and De Ceuninck F., eds), *Methods Mol. Med.* 100, 165–182, Humana Press
30 Lambrecht, S. *et al.* (2008) Differential proteome analysis of normal and osteoarthritic chondrocytes reveals distortion of vimentin network in osteoarthritis. *Osteoarthritis Cartilage* 16, 163–173
31 Yamagiwa, H. *et al.* (2003) Two-dimensional gel electrophoresis of synovial fluid: method for detecting candidate protein markers for osteoarthritis. *J. Orthop. Sci.* 8, 482–490
32 Gobezie, R. *et al.* (2007) High abundance synovial fluid proteome: distinct profiles in health and osteoarthritis. *Arthritis Res. Ther.* 9, R36
33 Katano, K. *et al.* (2009) Implication of GM-CSF induced neutrophil gelatinase-associated lipocalin in pathogenesis of rheumatoid arthritis revealed by proteome analysis. *Arthritis Res. Ther.* 11, R3
34 Kamphorst, J.J. *et al.* (2007) Profiling of endogenous peptides in human synovial fluid by nano LC–MS: method validation and peptide identification. *J. Proteome Res.* 6, 4388–4396
35 Steinbeck, M.J. *et al.* (2007) Myeloperoxidase and chlorinated peptides in osteoarthritis: potential markers of the disease. *J. Orthop. Res.* 25, 1128–1135
36 Jmeian, Y. and El Rassi, Z. (2008) Micro-high performance liquid chromatography platform for the depletion of high-abundance proteins and subsequent on-line concentration/capturing of medium-and low-abundance proteins from serum. Application to profiling of protein expression in healthy and osteoarthritis sera by 2-D gel electrophoresis. *Electrophoresis* 29, 2801–2811
37 Ling, S.M. *et al.* (2009) Serum protein signatures detect early radiographic osteoarthritis. *Osteoarthritis Cartilage* 17, 43–48
38 Xiang, Y. *et al.* (2004) Proteomic surveillance of autoimmunity in osteoarthritis. Identification of triosephosphate isomerase as an autoantigen in patients with osteoarthritis. *Arthritis Rheum.* 50, 1511–1521
39 Xiang, Y. *et al.* (2006) Fibulin-4 is a target of autoimmunity predominantly in patients with osteoarthritis. *J. Immunol.* 176, 3196–3204
40 Kato, T. *et al.* (2004) Neoantigens in osteoarthritic cartilage. *Curr. Opin. Rheumatol.* 16, 604–608
41 Hulejova, H. *et al.* (2007) Increased level of cytokines and matrix metalloproteinases in osteoarthritic subchondral bone. *Cytokine* 38, 151–156
42 Rollin, R. *et al.* (2008) Differential proteome of bone marrow mesenchymal stem cells from osteoarthritis patients. *Osteoarthritis Cartilage* 16, 929–935
43 Hitchcock, A.M. *et al.* (2008) Comparative glycomics of connective tissue glycosaminoglycans. *Proteomics* 8, 1384–1397