Osteoarthritis and Cartilage 18 (2010) 500–509

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Review

Proteomics role in the search for improved diagnosis, prognosis and treatment of osteoarthritis

C. Ruiz-Romero, F.J. Blanco*

Laboratorio de Investigación Osteoarticular y del Envejecimiento, Unidad de Proteómica-Nodo Asociado a ProteoRed-(Genoma España), Centro de Investigación Biomédica, Servicio de Reumatología, Complejo Hospitalario Universitario de A Coruña, 15006-A Coruña, Spain

ARTICLE INFO

Article history:
Received 1 June 2009
Accepted 23 November 2009

Keywords:
Osteoarthritis
Cartilage
Proteomics
Biomarkers
Chondrocytes

SUMMARY

Objective: Osteoarthritis (OA) is the most common rheumatic pathology. It is related to aging and is characterized primarily by cartilage degradation. Despite its high prevalence, the diagnostic methods currently available are limited and lack sensitivity. The focus of this review is the application of proteomic technologies in the search of new biomarkers for improved diagnosis, prognosis and treatment of OA.

Methods: This review focuses on the utilization of proteomics in OA biomarker research to enable early diagnosis, improved prognosis and the application of tailored treatments.

Results: New diagnostic tests for OA are urgently needed and would also promote the development of alternative therapeutic strategies. Considering that OA involves different tissues and complex biological processes, the most promising diagnostic approach would be the study of combinations of biomarkers. New experimental approaches for the identification and validation of OA biomarkers have recently emerged and include proteomic technologies. These techniques allow the simultaneous analysis of multiple markers and become a very powerful tool for both biomarker discovery and validation.

Conclusions: Improvements in proteomics technology will undoubtedly lead to advances in characterizing new OA biomarkers and developing alternative therapies. Even so, further work is required to enhance the performance and reproducibility of proteomics tools before they can be routinely used in clinical trials and practice.

Introduction

Osteoarthritis (OA) is the most frequent arthropathy. It is characterized by progressive degradation of hyaline articular cartilage and is associated with aging. Prevalence studies show that OA usually develops after age 45, its frequency increases with age, and it affects more than 10% of the population. It is the leading cause of permanent work incapacity and one of the most common reasons for visiting primary care physicians. As the population ages, it is estimated that the number of people with some degree of OA will double over the next three decades.

A major objective for OA research is the conceptualization and development of early diagnostic strategies. OA is clinically silent in most individuals during its initial stages and extensive deterioration of cartilage already exists by the time of diagnosis. Currently, the diagnosis of OA relies on the description of pain symptoms, stiffness in the affected joints, and radiography, used as the reference technique for determining the grade of joint destruction. Limitations in diagnostic tests presently available provide impetus for the substantial increase in interest in finding new specific biological markers of cartilage degradation, both to facilitate early diagnosis of joint destruction and to enhance disease prognosis and evaluation of progression.

In the recent years, new strategies for OA biomarker discovery and validation have emerged. These include genomic, proteomic and metabolomic technologies. The current strategy most employed is transcriptomic analysis using DNA microarrays, allowing identification of candidate genes possibly involved in cartilage degradation[1,2]. Gene expression levels, however, do not necessarily predict protein levels because of alternative transcriptional and translational steps, and the activity of protein degradation processes. Moreover, genomic studies do not take into account post-translational modifications (PTMs) of proteins or their interactions, in many cases essential for biological activities. The advantage of proteomics is that the actual functional molecules of

* Address correspondence and reprint requests to: Francisco J. Blanco, Laboratorio de Investigación, Servicio de Reumatologia, Complejo Hospitalario Universitario de A Coruña, 15006-A Coruña, Spain. Tel: 34-981-178272; Fax: 34-981-178273.

E-mail address: francisco.blanco.garcia@sergas.es (F.J. Blanco).

1063-4584/$ – see front matter
doi:10.1016/j.joca.2009.11.012

the cell are studied; presenting a true picture of what is occurring in the tissue, not what might be happening. We have recently reviewed those proteomic analyses performed to increase knowledge about OA pathogenesis[3]. In this review, we will focus on the utility of proteomic approaches for OA biomarker research. Proteomic technologies currently available will be discussed, with particular emphasis on their application to the search of new diagnostic, prognostic and therapy markers for OA.

Proteomics strategies and technology

Proteomics is a research tool for the large-scale study of protein structures, functions and interactions. Unlike the genome, the proteome is highly dynamic, responding to environmental changes, stress, pathological situations, drug administration, etc. These factors increase proteome complexity through activation or suppression of gene expression, alterations in protein interactions, or changes in PTMs, all affecting protein function.

The basic scheme for proteomics relies on the separation of a large number of proteins and their identification by mass spectrometry (MS). Methods and protocols for these techniques are available in review articles and laboratory handbooks[4,5]. The selection of a proteomic approach is mainly dependent on the technology that the researcher has available (specially, the type and sensitivity of the mass spectrometer). Each of the strategies that can be followed for proteomic biomarker discovery or validation has advantages and disadvantages that are showed in Table I. In many cases they are not overlapping techniques and provide complementary information.

Protein separation strategies in proteomics

The separation strategy is a critical step in proteomics. The first proteomic separation methods have been carried out by two-dimensional electrophoresis (2-DE) of the proteins. These techniques separate proteins by their isoelectric point in the first dimension (using immobilized pH gradient (IPG) strips), and then by their molecular weight in the second dimension (using conventional SDS-PAGE gels). The gels are then stained by various techniques[6–8] to visualize the protein spots. Gel images are digitized for analysis, and the proteins selected for identification are picked from the gels and identified by MS[9]. Gel-based strategies have several advantages over other separation techniques, particularly their high resolution and their capacity for the direct

Table I
Proteomic strategies in biomarker research

Strategy	Advantages	Disadvantages	Power*
Shotgun proteomics (survey approach, relative quantification)			
2-DE, 2D DIGE	• High resolution • Direct detection of PTMs • Information about Mw and pI of the protein	• Low throughput • Limited dynamic range • Low-abundant proteins are masked • Not the technique of choice with biological fluids	* BD
Differential labeling and LC–MS/MS (ICAT, iTRAQ, 18O, SILAC…)	• Medium throughput • Easy automation • High resolution with 2D or 3D separations • Higher quantification accuracy than label-free methods	• Peptide to protein inference problems • High labeling costs • Limited number of experiments to be compared	**/*** BD
Label-free LC–MS/MS	• Medium throughput • Easy automation • High resolution with 2D or 3D separations • Avoids time and cost-consuming labeling step • Unlimited number of experiments can be compared	• Peptide to protein inference problems • Less accurate than labeling-based MS methods	**/*** BD
SELDI-TOF-MS	• High throughput and automation	• Proteins are not identified • Lack of any biological information	** BD, BV
Protein arrays	• High throughput and automation • Array format • Versatility • High multiplexing power	• Need specific instrumentation • Array development • Sensitivity limited to fluorescence signal	**/*** BD, BV
Targeted proteomics (candidate-based approach)			
AQUA	• High throughput and automation • High sensitivity and accuracy • Allow absolute quantification	• Cost of SIL peptides for each protein • Chemical synthesis of SIL peptides not always feasible	** BV
QconCAT	• High sensitivity and accuracy • Low cost • Avoids chemical synthesis • Allow absolute quantification	• Time-consuming QconCAT design and biosynthesis	** BV
MRM-MS	• High sensitivity and accuracy • High throughput • High multiplexing power • Absolute quantification	• MRM transitions design may be complex • Performed on triple quadrupole MS instruments	*** BV
SISCAPA	• Enrichment of the target peptides • High sensitivity and accuracy • Absolute quantification	• Need of specific antibodies • Essential immunoaffinity step	** BV
Protein arrays	• High throughput and automation • Array format • Versatility • High multiplexing power	• Need specific instrumentation • Array development • Sensitivity limited to fluorescence signal	**/*** BD, BV

Mw: molecular weight, pI: isoelectric point.
***: highly suitable, **: suitable, *: suitable with limitations.
* Utility of the approach for: BD: biomarker discovery, or BV: biomarker verification/validation.

detection of PTMs of proteins. On the other hand, drawbacks of this technique are its poor capacity for high-throughput analysis, the difficulty to separate particular proteins (either very acidic/basic or very small/large), and that its dynamic range is dependent on gel staining, which may interfere with protein identification.

On the other hand, gel-free or liquid chromatography (LC) separation methods are currently acquiring greater importance. Technological advances in this field provide the advantages of being more easily automated, allowing higher throughput and having increased capacity to detect low-abundant proteins usually masked by the most abundant proteins in two-dimensional (2D) gels. Chromatographic separations usually require in-solution digestion of proteins before analysis; thus, peptides are the molecules resolved. Like gel-based techniques, chromatographic processes may involve one or more separation steps[10,11]. In all these techniques, protein analysis and identification both use MS data; therefore, these strategies are also known as MS-based proteomics[9].

MS technology and strategies used in proteomics

MS protein identification involves a series of steps for protein or peptide ionization, ion separation, and detection that varies according to the instrumentation used. Ionization of peptides or proteins present in the sample may be carried out in solid state when using instruments with a *matrix-assisted laser desorption/ionization* (MALDI) source or in solution for electrospray ionization (ESI) sources (Fig. 1). Ions are separated according to their mass/charge relationship (*m/z*), usually using a time-of-flight (TOF), quadrupole or ion trap analyzer. Following this step, ion masses are measured in a detector to provide a mass spectrum reflecting the ion abundancevs its *m/z* value used for protein identification. Latest advances in instrumentation, with the introduction of Fourier transform ion cyclotron resonance (FT-ICR)-MS[12] and the most recently introduced mass analyzer Orbitrap[13], provide the advantage of much high resolution, mass accuracy and sensitivity, and are now being used more frequently for biomarker identification.

In proteomics, samples are subjected to multi-dimensional separation techniques, and individual protein spots or fractions are digested with an endoprotease (usually trypsin) to yield peptide fragments. Then, the peptides are analyzed by MS to establish their identity, based on their peptide mass fingerprints or further mass fragmentation to obtain sequence information. Peptide mass fingerprinting (PMF) identification (Fig. 1) is accomplished on proteins that are previously isolated (either by protein purification or 2-DE), by entering the experimental masses of tryptic peptides into a database search of predicted protein masses. Protein sequence identification is performed by determination of the amino acid sequence of a protein[14,15].

Quantitative proteomics approaches

Quantification of protein levels, in order to achieve accurate differential protein profiling between samples, has been a major challenge in proteomics[16]. The best quantitative gel-based technique to date is differential in-gel electrophoresis (DIGE), in which protein samples are labeled with fluorescent tags before mixing them and running them on a 2D gel with a pooled sample used as the internal standard for quantification[17]. This technique reduces inter-gel variation and false positives[18,19] and results in reliably reproducible data with biological significance. Nevertheless, given its limitations regarding short dynamic range and poor detection of low-abundant proteins, gel-based techniques are now considered inappropriate for biomarker discovery or validation, as these experiments are usually carried out on plasma or other body fluids that present a high dynamic range and a number of high abundant proteins that mask the rest (Table I).

For LC–MS quantification, differential labeling of the samples prior to analysis is usually required (Table I). Several *in vitro* chemical or enzymatic labeling methods are used, such as isotope coded affinity tag (ICAT)[20], isobaric tag for relative and absolute quantitation (iTRAQ™)[21] or ^{18}O[22]. Other *in vivo* metabolic labeling procedures have been developed and consist of culturing cells in media with a 'heavy' amino acid[23,24] ^{15}N or ^{13}C[25], and comparing them to cells grown in media containing unlabeled 'light' amino acids. Regardless of the labeling method, quantification is achieved by comparing MS peak intensities of a partner pair of heavy and light peptides. Recent advances in software applications have also

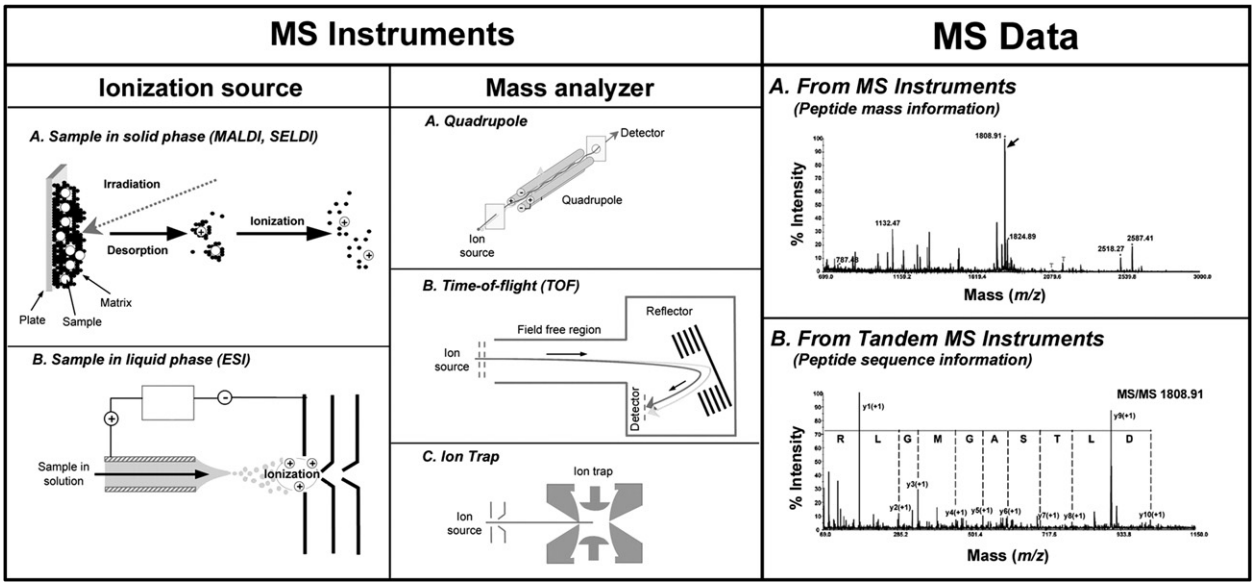

Fig. 1. MS in proteomics. Left, MS instruments employed in proteomics are characterized by their type of ionization source and their type of mass analyzer/s. Right, MS information obtained from these instruments. Devices with two or more analyzers are able to provide mass and sequence information of the peptides, whereas those with one analyzer give only mass information and allow protein identification only by peptide mass fingerprinting.

been developed for protein quantification in mixtures without electrophoresis or isotope labeling[26].

The techniques described thus far provide relative quantification information by measuring the relative abundance ratio between two or more samples. Absolute protein quantification methods in proteomics attempt to measure the absolute protein level by introducing a characteristic peptide unique to a specific protein, providing an explicit external standard of known concentration against which to measure the protein level[27]. In the AQUA technique (Table I), peptides are synthesized incorporating stable isotopes to provide a known mass offset, and these peptides are used as an internal standard[28]. Similarly, the quantification concATamers (QconCAT) method concatenates stable isotope labeled (SIL) peptides into a recombinant protein, which is synthesized in bacterial cell cultures[29,30]. In many of these analyses, quantification is achieved by multiple reaction monitoring (MRM) MS assays. These experiments use ESI of the peptides followed by two stages of mass selection, the first one selecting the mass of the intact peptide (parent ion) and the second one a specific fragment of the parent (MRM transitions)[31]. MRM assays coupled with isotope dilution MS have proved their utility for quantitative analysis of biomarkers[32,33]. Moreover, their reproducibility, recovery, linear dynamic range, limits of detection (down to ng/ml) and quantification of protein biomarkers in plasma have been recently assessed[34]. Therefore, MS analysis driven in quantitative MRM mode is now appearing as a promising alternative to quantify proteins in biological fluids, avoiding the need of specific antibodies and enabling multiplexed analysis (of more than 100 proteins simultaneously). Finally, the stable isotope standards and capture by anti-peptide antibodies (SISCAPA) approach[35] exploits the use of immobilized anti-peptide antibodies, used to isolate specific peptides together with stable isotopically labeled versions of the same peptides prior to MRM analysis.

Protein chips

Chip technology is beginning to be applied in proteomics. Differentially to what happens in genomics, a simple chip for all proteins is not currently achievable due to the high heterogeneity of proteins. However, a variety of protein and peptide arrays have been developed for analyzing a specific protein or group of proteins. Surface-enhanced laser desorption ionization (SELDI) interfaces coupled to a TOF analyzer have made specific protein quantification in clinical proteomics a reality[36–38], and have been used for clinical and biomedical systems studies, employing different body fluids to search for biomarkers. Nevertheless, it does not give any biological information, as the signature peaks are not identified.

More recently, other protein microarray platforms are being developed[39]. There are currently two general types, analytical microarrays and functional protein microarrays (Fig. 2)[40]. Analytical microarrays are designed for quantitative assessment of potential biomarkers and other useful proteins. They are most commonly forward-phase arrays where antibodies or antibody mimics are immobilized on a solid surface and exposed to a test sample containing a mixture of proteins. Detection is usually accomplished using labeled samples or a secondary antibody that recognizes the antigen of interest (multiple sandwich immunoassays). Reverse phase analytical arrays have been developed that are essentially reciprocals of this technique to profile antibodies in the plasma or serum[41]. Also, functional microarray chips to immobilize purified peptides or whole proteins on a small surface have been developed. Unlike antibody microarrays, which are mainly used for diagnostics and protein expression profiling, functional protein arrays are used for the study of biochemical activities, PTMs, drug-target identification, and to analyze protein–protein, DNA–protein, RNA–protein, and drug–protein interactions.

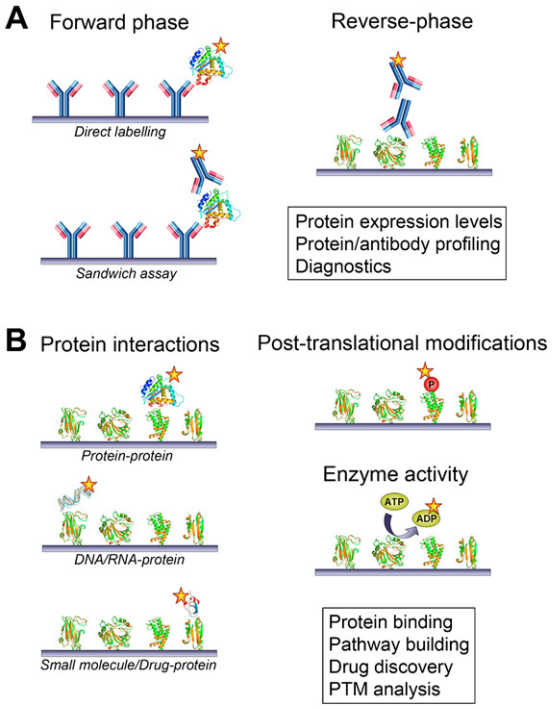

Fig. 2. Types of protein arrays. A: Analytical arrays. B: Functional arrays. Protein arrays will become the approach of choice for closing the information gap between genomics and proteomics for the development of new disease biomarkers and therapies.

Proteomics in the search for OA biomarkers

The use of proteomics in biomarker discovery and validation

Proteomics has produced great expectations for the discovery of biomarkers to improve the diagnosis of a wide range of diseases. Biomarker research involves a series of steps moving from discovery to the launch of a commercial biomarker product (Fig. 3). The technologies of proteomics and metabolomics have a huge potential for both biomarker discovery and verification or validation. There are two general approaches, target specific and global/non-directed, for proteomic biomarker discovery. Target-specific approaches frequently use antibodies to screen specific proteins by western blot analysis, enzyme-linked immunosorbent assay (ELISA), or antibody arrays, and are useful for validation and clinical studies.

However, global/non-directed approaches may have more potential for biomarker discovery because they are unbiased, high-throughput screens. There are also two strategies for non-directed approaches, techniques that profile unidentified proteins and those that generate patterns of identified proteins[42]. Profiling of unidentified proteins often, though not always, utilizes MALDI-TOF-MS or SELDI-TOF-MS. The main advantage of these techniques is speed in processing many samples, making them attractive tools for clinical screening. Because peptides of interest are not identified in SELDI, validation using other techniques is difficult. However, by using additional steps after MALDI-TOF analysis, protein peaks of interest can be identified. A list of identified proteins is typically obtained by tandem MS approaches. Although protein identification is now much faster than before human genome sequencing, it is still slower than the profiling-based proteomic procedures

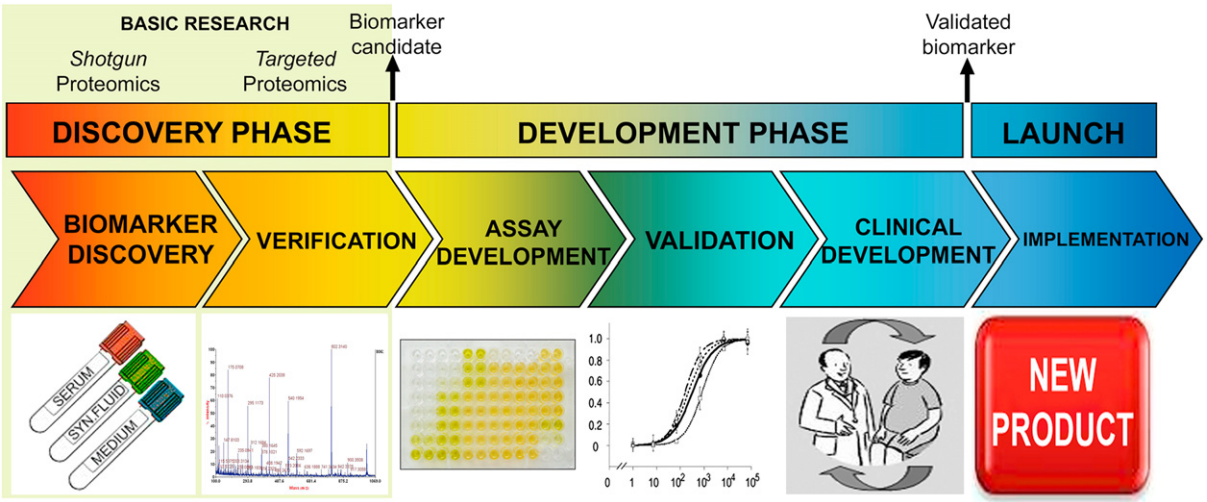

Fig. 3. Steps in biomarker research.

mentioned above that do not rely on peptide fragmentation or database searches to identify peaks of interest, but still provide much information.

Blood (plasma and/or serum), and other body fluids are expected to be excellent sources of protein biomarkers for proteomic analyses because they come in contact with most tissues. During this contact, body fluids pick up proteins secreted or shed by tissues, a hypothesis that has been recently confirmed[43]. Although the major advantage of using plasma and/or serum is that it is readily obtained, proteins secreted or released from a specific tissue or cell type – those that hold the highest potential as biomarkers – are diluted in blood to a degree that frequently makes them undetectable by the current available methods. Therefore, great interest has also been focused on analysis of the so-called "proximal" body fluids, those which contact only one or a few tissues, thus less dilution of tissue-derived proteins would be expected. Table I illustrates proteomic strategies for finding new biomarker molecules in human body fluids or conditioned media in OA research.

Proteomic analysis of plasma and serum

Human plasma has been termed the most complex human proteome[44]; its extraordinarily high dynamic range of concentrations of individual proteins challenges current proteomics technology. Moreover, large variations have been shown to exist between individuals in the concentration and state of modification of some plasma proteins[45], creating a requirement for analyzing samples from a large number of individuals for statistical accuracy.

Disease biomarkers typically appear at low concentrations in plasma, making their detection difficult due to the presence of higher abundance proteins. A practical and effective strategy to solve this problem is the removal of diagnostically uninformative high abundance proteins to enhance the detection of low abundance proteins and penetrate deeper into the plasma proteome. Several systems for plasma protein depletion have been developed and are commercially available. They consist of antibody-based resins with affinities to as many as 20 of the most abundant plasma proteins[46], whose removal improves the ability to identify lower abundance proteins. Disadvantages of this needed depletion step include reproducibility problems over large samples sets (the half-life of a depletion column does not exceed 150–200 runs), and the fact that some proteins of interest might be non-specifically depleted due to interactions with other proteins such as albumin.

One of the first proteomic studies of the serum of OA patients was based on the hypothesis that immunological pathways may be implicated in the pathophysiology of OA. The authors developed a procedure to identify autoantibodies and compared their levels in sera from OA and rheumatoid arthritis (RA) patients[47]. In this work, a 2-DE-based strategy was followed by MALDI-TOF-MS, allowing recognition of autoantigens by specific autoantibodies present in serum (Table II). Anti-triose phosphate isomerase (TPI) protein autoantibodies were found in OA patients; their presence was associated with lower radiographic grades of disease. Using the same methodology, the authors also identified autoantibodies to fibulin-4 in the serum of patients with OA[48]. Currently, SELDI-TOF-MS is employed for identification of new biomarkers specific for RA[49], using OA samples as a non-inflammatory control group. Proteomic analyses of blood cells from RA patients have also been performed[50,51]. Although this approach has not yet been applied in OA research, it could present a new opportunity.

Finally, using MRM MS and SIL synthetic peptides as internal standards, C-reactive protein (CRP, a diagnostic marker of RA) was detected in serum samples taken from patients with either erosive or non-erosive RA and compared to healthy individuals[52]. This exemplifies the potential of these targeted proteomics approach for OA biomarker quantitative studies on serum samples.

Proteomic analysis of synovial fluid (SF)

The specific features of OA require highly sensitive methods for biomarker identification. The use of SF rather than serum for proteomic techniques to search for biomarkers of OA is advantageous because it avoids their dilution in other biological fluids. SF is a logical potential compartment for OA biomarkers because it is derived directly from the diseased site and functions in the exchange of proteins between articular cartilage and the systemic circulation. Consequently, many proteomic strategies in rheumatology are designed to identify putative biomarkers in SF before their validation in serum.

SF sampling is invasive and samples may need to be clarified by centrifugation to remove contaminating cells, such as mononuclear cells, by centrifugation before use in proteomics. It is also recommended to treat SF samples with hyaluronidase to digest the hyaluronic acid in order to facilitate proteomics analysis[53]. Figure 4

Table II
Proteomic approaches in the search for OA biomarkers

Source	Goal of the analysis	Proteomic strategy	Proteins related to disease or treatment	References
Body fluids				
SF	RA vs OA	SELDI	MRP-8	Uchida *et al.*[60]
SF and plasma	RA vs OA	2-DE	Calgranulin A, Serum amyloid A, Fibrinogen	Sinz *et al.*[54]
SF	OA inter-sample analysis	2-DE	Haptoglobin	Yamagiwa *et al.*[53]
SF and plasma	RA vs OA	2-DE	MRP-8, MRP-14	Drynda *et al.*[55]
Serum	RA vs OA, PsA, Asthma, Crohn's and healthy	SELDI	MRP-8	de Seny *et al.*[49]
Urine	OA metabolic profiling	RMN	Metabolite OA profile	Lamers *et al.*[67]
SF	Early and late OA vs control	1-DE + LC–MS/MS	18 altered in OA	Gobezie *et al.*[58]
SF	OA vs control	UF + LC–MS/MS	COL2, PRG4, SAA, TUB, VIME, MGP	Kamphorst *et al.*[59]
Urine	OA vs control	Immunoaffinity LC–MS/MS	uTIINE	Nemirovskiy *et al.*[66] Li *et al.*[65]
Secreted proteins (conditioned media)				
Cartilage	OA vs control	2-DE + LC–MS/MS	Activin A, COL2, TIMP	Hermansson *et al.*[68]
Cartilage	OA vs control	2-DE + off-gel + antibody arrays	PEDF, SAP, OGN, YKL-39, Gelsolin, TIMP-1	De Ceuninck *et al.*[69]
Cartilage	Effect of cytokines or compression	1-DE + LC–MS/MS	58 altered by treatment (COMP, COL6, SAA…)	Stevens *et al.*[70]
Cartilage	Effect of IL-1β or RetA	2-DE	20 altered by treatment (COMP, Matrilin-3…)	Wilson *et al.*[71]
Chondrocytes	Effect of IL-1β or TNF-α	Antibody array	IL-8, IL-6, OPG	De Ceuninck *et al.*[72]
Chondrocytes	Effect of IL-1β or OSM	2-DE	MMP-1 and -3, YKL-40, Cofilin, Cyclophylin A	Catteral *et al.*[73]
Chondrocytes	Effect of LPS	1-DE + LC–MS/MS	YKL-40, MMP-3 and -13, OGN	Haglund *et al.*[74]

1-DE: one-dimensional electrophoresis; COL2: type II collagen; COL6: type VI collagen; MGP: matrix Gla protein; MS/MS: tandem mass spectrometry; OGN: osteoglycin; OPG: osteoprotegerin; OSM: oncostatin M; OSTP: osteopontin; PARC: pulmonary and activation regulated chemokine; PEDF: Pigment epithelium-derived factor; PRG4: proteoglycan 4; PsA: psoriatic arthritis; RetA: all-*trans*-retinoic acid; RMN: nuclear magnetic resonance; SAA: serum amyloid A; SAP: serum amyloid P; UF: ultrafiltration; uTIINE: urinary type II collagen neoepitope; TUB: tubulin; VIME: Vimentin.

shows a representative 2-DE gel of human SF. As occurs with serum, high quantitative inter-sample variability has been reported for a number of proteins using 2-DE on samples from OA patients[54]. Many proteomic studies performed on SF have focused on RA and use samples of SF from OA knees for controls (Table II). Differential analysis of 2-DE protein patterns of SF from OA and RA patients have enabled the identification of molecular markers specifically related to RA rather than to OA[55], such as calgranulin B and A amyloid protein. The same authors employed a similar approach to identify the S100A8/A9 heterocomplex as a marker for discriminating RA from OA[56]. LC-based approaches were used to study SF and serum from RA or OA patients and the results revealed a high number of putative RA biomarkers[57]. Another study identified various possible prognostic RA biomarkers in SF and validated them in serum[53]. More recently, two different proteomic approaches have been developed to gain knowledge of the OA SF protein profile. One methodology focused on the high abundance proteome[58], using one-dimensional (1D) PAGE followed by LC–MS/MS analysis to identify 18 proteins whose concentrations were different in OA samples than in controls. Another experiment studied SF endogenous peptides using ultrafiltration and LC–MS/MS analysis[59]. These authors suggested six proteins that are potentially useful as markers for the diagnosis of OA (Table II). Finally, the use of protein biochips with SELDI-MS led to the finding

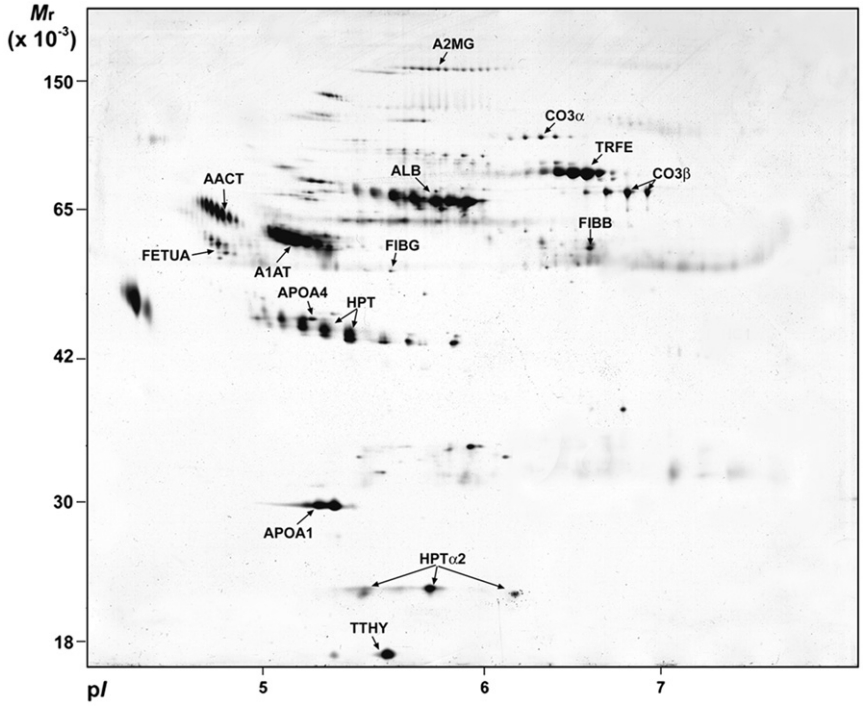

Fig. 4. Proteomic map of human SF. Separation of SF proteins by 2-DE allows their visualization and permits a comparative study of OA and normal samples. MS identification of the differentiated proteins completes the analysis.

of several reproducible and discriminatory biomarker candidates for distinguishing between RA and OA[60].

Proteomic analysis of other body fluids

Urine, saliva and tears are easily obtained by non-invasive procedures. Of these, urine is most often used for OA biomarker investigations. Currently available tests measure the levels of specific proteins in urine, and assays for determining the presence of cartilage degradation markers in urine have been developed. These include type II collagen C-telopeptide fragments (CTX)[61,62] and the collagenase cleavage site neoepitope (TIINE)[63]. Recently emerging proteomic technologies now permit the simultaneous examination of the patterns of multiple urinary proteins and their correlation to individual diagnoses, responses to treatment or prognoses (reviewed in Ref. 64). A targeted proteomics approach has been developed for the quantification of urinary TIINE (uTIINE) using immunoaffinity LC–MS/MS[65,66], and a metabolomic urinary profile associated with OA has been identified[67].

Analysis of cartilage or chondrocyte secretomes

The secretome refers to the global array of proteins secreted by a cell, a tissue or an organism. It is a potential source for the discovery of biomarker candidates because secreted molecules are released into the extracellular space and should be detectable in body fluids. The first analysis of the OA cartilage secretome used 2-DE and LC–MS/MS[68]. These authors found increased type II collagen synthesis in OA cartilage media and identified new potential regulatory molecules of cartilage turnover, such as activin A. Another study evaluated different technologies for determining the OA cartilage secretome, including 2-DE, off-gel electrophoresis and antibody microarrays[69]. This study identified 43 proteins secreted from OA cartilage, some of which were proposed as potential candidates for biomarkers of diseased cartilage (Table II). More recently, a study compared secretome profiles from bovine cartilage explants treated with interleukin-1β (IL-1β) or tumor necrosis factor α (TNF-α) or subjected to traumatic mechanical compression[70]. This study found that cytokines stimulate cartilage cells to release proteins associated with innate immune and stress responses, whereas the overload compression injury induced the release of those typical intracellular proteins seen with cartilage integrity loss, including matrix damage and cell membrane disruption. The effect of IL-1β and retinoic acid on cartilage secretomes was also evaluated by a 2-DE approach[71] from which the investigators identified 20 proteins altered by one or both treatments, including matrix metalloproteinase (MMP)-3, cartilage oligomeric matrix protein (COMP), matrilin-3 or gelsolin.

An alternate approach analyzed the secretome from cultured chondrocytes. Media from normal and OA chondrocytes treated with the proinflammatory cytokines IL-1β and TNF-α were incubated with array membranes holding 79 antibodies directed against cytokines, chemokines, and angiogenic and growth factors[72]. Using this technique, seven altered proteins were identified (Table I), all of which were also found to be secreted by OA cartilage[69]. This finding validates the usefulness of studying the chondrocyte secretome as a model of events occurring in cartilage tissue. The secretome of human articular and bovine nasal chondrocytes stimulated by IL-1β and oncostatin M was also derived using 2-DE; proteins regulated differently by these agents were identified by ESI-MS/MS[73]. This work discovered that some of the secreted proteins were cleaved into smaller fragments by proteolysis. This finding illustrates the valuable information that proteomics approaches can provide about protein processing, which cannot be provided by gene-based arrays. Also, a comparative proteomic analysis using 1D-PAGE and LC–MS/MS was performed on proteins secreted from lipopolysaccharide (LPS)-stimulated rat articular chondrocytes in order to study LPS-induced stress responses of articular cartilage[74].

Future perspectives

The genome has been the primary focus of past research on the molecular basis of disease. Proteomics bridges the gap between what is encoded in the genome and its translation into proteins, complementing genomics-based approaches and providing additional information. Recent improvements in proteomics technologies allowed its application in the clinical field, which may lead to a better understanding of disease biology, the identification of biomarkers and the development of new therapeutic strategies. New protein array formats and advances in MS equipment have been major contributions[75], although a number of serious challenges are still being faced, including achieving higher sensitivity and dynamic range of detection methods and strategies for the study of PTMs.

An emerging technique for the discovery of protein profiles involves the identification of biomarkers by MALDI MS directly on tissue biopsies[76]. Traditionally, imaging MS (IMS) had required substantial effort for sample preparation and data analysis, rendering it unsuitable for routine clinical use. Recently, IMS techniques have been optimized[77] and a newly described procedure allows imaging of formalin-fixed, paraffin-embedded tissue

Table III
Putative OA protein biomarkers described by proteomic strategies

Source	Marker*	Protein name	BIPED†	Ref.
Body fluids				
SF and plasma	MRP-8, -14	Myeloid-related proteins (Calgranulins S100)	I	49,55,60
	SAA	Serum amyloid A protein	I	54,59
	FIB	Fibrinogen fragments	P, E, D	54,58
	HPT	Haptoglobin	I	53
SF	COL2	Type II collagen fragments	P, E, D	59
	PRG4	Proteoglycan 4	P, E, D	59
	TUB	Tubulin	I	59
	VIME	Vimentin	I	59
	MGP	Matrix gla protein	I	59
Urine	uTIINE	Type II collagen neoepitope	B, P, E	65,66
Secreted proteins				
Cartilage	COL2	Type II collagen fragments	P, E, D	68
	COL6	Type IV collagen fragments	I	70
	COMP	Cartilage oligomeric matrix protein	D, B, P	71
	INHBA	Activin A (Inhibin beta A chain)	I	68
	GELS	Gelsolin	P, E, D	69
	HPT	Haptoglobin	I	70
	MAT3	Matrilin-3	I	71
	OGN	Osteoglycin (Mimecan)	P, E	69,74
	SAA	Serum amyloid A protein	I	70
	SAP	Serum amyloid P protein	I	69
Chondrocytes	MMP-1, -3, -13	Metalloproteinases	B, P, E	73,74
	OGN	Osteoglycin (Mimecan)	P, E	69,74
	OPG	Osteoprotegerin	B, P, E	72
	TIMP-1	Tissue inhibitor of Metalloproteinase-1	B, P	69
	YKL-39, -40	Chitinase-like family of proteins	I	69,73

Abbreviations: B, burden of disease; I, investigative; P, prognostic; E, efficacy of intervention; D, diagnostic.

* Proteins identified in the proteomic studies listed and referenced in Table I.

† Hypothetical BIPED classification of the markers[80].

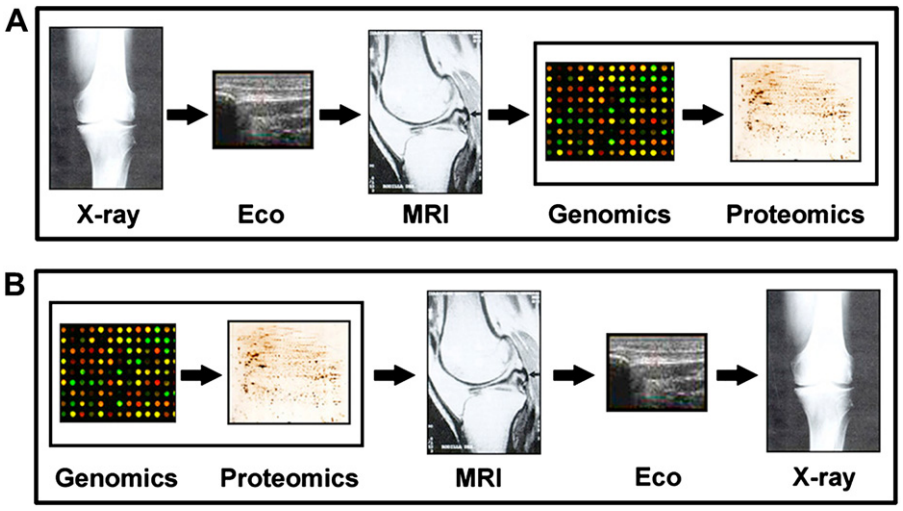

Fig. 5. Panel A: Chronological appearance of images in the diagnostic process of a rheumatic disease. Panel B: Sequence of the information provided from the different images from the origin of the disease. Both genomics and proteomics have produced new images to aid physicians in making an early diagnosis and choosing an individually tailored therapy for patients with OA. The understanding and correct interpretation of these images will become part of daily rheumatology practice in the near future. Interestingly, although these images have been the last to appear, they provide information about what is happening to patients or populations in the earliest stages of rheumatic diseases. Therefore, these tests and images will soon be utilized before radiographic imaging in the OA diagnosis process. We will move from managing radiological images from rheumatic diseases to molecular pictures, obtained either from genomics or proteomics.

sections[78], thus providing access of this technique to large numbers of archived samples in clinical pathology. The ability of MALDI-IMS to determine the distribution in a certain tissue of hundreds of unknown compounds in a single measurement makes it a powerful technique to achieve a better understanding of the underlying molecular mechanisms of disease. On the other hand, protein array-based strategies have gained in popularity for clinical sample evaluation because of their high throughput and automation level. Protein chips are currently used for large-scale screening of libraries to identify novel targets or drugs, and probably have the most potential for analyzing a set of known OA biomarkers.

While these improvements in proteomics technology will undoubtedly lead to advances in characterizing new OA biomarkers, further work is required to enhance the performance and reproducibility of proteomics tools before they can be routinely used in clinical trials and practice. Up to date, proteomic tools have a huge power for biomarker discovery (Fig. 3), as they have already identified more than a hundred of proteins (or protein fragments) that might be related with OA (Table II). Some of these, such as COMP, Coll2 or MMPs were previously detected in other studies and are currently being validated as OA biomarkers, whereas others have been characterized only in proteomic analyses, and might be subjected to further verification assays (Table III).

Taking into account its complex pathophysiology, systems biology approaches to OA – with which researchers try to understand the disease from the level of molecular pathways and the structure and dynamics of regulatory networks[79] – are likely difficult to achieve. "*Omics*" strategies provide a huge amount of experimental data, and efforts are now required to integrate this information into an understanding of the joint system and its derangement in OA. When these hurdles are overcome, inclusion of proteomics in daily diagnosis, prognosis and therapy response monitoring of OA patients will become a reality (Fig. 5), opening the gateway for personalized molecular medicine with early stage diagnosis and tailored therapies designed according to the protein profile of each individual patient.

Conflict of interest

Authors have not any conflict of interest.

References

1. Aigner T, Saas J, Zien A, Zimmer R, Gebhard PM, Knorr T. Analysis of differential gene expression in healthy and osteoarthritic cartilage and isolated chondrocytes by microarray analysis. Methods Mol Med 2004;100:109–28.
2. Marshall KW, Zhang H, Nossova N. Chondrocyte genomics: implications for disease modification in osteoarthritis. Drug Discov Today 2006;11:825–32.
3. Ruiz-Romero C, Blanco FJ. The role of proteomics in osteoarthritis pathogenesis research. Curr Drug Targets 2009;10:543–56.
4. Cox J, Mann M. Is proteomics the new genomics? Cell 2007;130:395–8.
5. Veenstra TDIn: Proteomics for Biological Discovery. New York: John Wiley & Sons; 2006;Vol. I
6. Neuhoff V, Arold N, Taube D, Ehrhardt W. Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with clear background at nanogram sensitivity using Coomassie Brilliant Blue G-250 and R-250. Electrophoresis 1988;9:255–62.
7. Shevchenko A, Wilm M, Vorm O, Mann M. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. Anal Chem 1996;68:850–8.
8. White IR, Pickford R, Wood J, Skehel JM, Gangadharan B, Cutler P. A statistical comparison of silver and SYPRO Ruby staining for proteomic analysis. Electrophoresis 2004;25:3048–54.
9. Aebersold R, Mann M. Mass spectrometry-based proteomics. Nature 2003;422:198–207.
10. Liu H, Lin D, Yates 3rd JR. Multidimensional separations for protein/peptide analysis in the post-genomic era. Biotechniques 2002;32:898, 900, 902 passim.
11. Washburn MP. Utilisation of proteomics datasets generated via multidimensional protein identification technology (MudPIT). Brief Funct Genomic Proteomic 2004;3:280–6.
12. Marshall AG, Hendrickson CL, Jackson GS. Fourier transform ion cyclotron resonance mass spectrometry: a primer. Mass Spectrom Rev 1998;17:1–35.

13. Hu Q, Noll RJ, Li H, Makarov A, Hardman M, Graham Cooks R. The Orbitrap: a new mass spectrometer. J Mass Spectrom 2005;40:430–43.
14. Hernandez P, Muller M, Appel RD. Automated protein identification by tandem mass spectrometry: issues and strategies. Mass Spectrom Rev 2006;25:235–54.
15. Nesvizhskii AI. Protein identification by tandem mass spectrometry and sequence database searching. Methods Mol Biol 2007;367:87–119.
16. Ong SE, Mann M. Mass spectrometry-based proteomics turns quantitative. Nat Chem Biol 2005;1:252–62.
17. Unlu M, Morgan ME, Minden JS. Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. Electrophoresis 1997;18:2071–7.
18. Lilley KS, Friedman DB. All about DIGE: quantification technology for differential-display 2D-gel proteomics. Expert Rev Proteomics 2004;1:401–9.
19. Marouga R, David S, Hawkins E. The development of the DIGE system: 2D fluorescence difference gel analysis technology. Anal Bioanal Chem 2005;382:669–78.
20. Gygi SP, Rist B, Gerber SA, Turecek F, Gelb MH, Aebersold R. Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. Nat Biotechnol 1999;17:994–9.
21. DeSouza L, Diehl G, Rodrigues MJ, Guo J, Romaschin AD, Colgan TJ, *et al*. Search for cancer markers from endometrial tissues using differentially labeled tags iTRAQ and cICAT with multidimensional liquid chromatography and tandem mass spectrometry. J Proteome Res 2005;4:377–86.
22. Miyagi M, Rao KC. Proteolytic ^{18}O-labeling strategies for quantitative proteomics. Mass Spectrom Rev 2007;26:121–36.
23. Ong SE, Blagoev B, Kratchmarova I, Kristensen DB, Steen H, Pandey A, *et al*. Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. Mol Cell Proteomics 2002;1:376–86.
24. Ong SE, Mann M. Stable isotope labeling by amino acids in cell culture for quantitative proteomics. Methods Mol Biol 2007;359:37–52.
25. Snijders AP, de Vos MG, de Koning B, Wright PC. A fast method for quantitative proteomics based on a combination between two-dimensional electrophoresis and ^{15}N-metabolic labelling. Electrophoresis 2005;26:3191–9.
26. Haqqani AS, Kelly JF, Stanimirovic DB. Quantitative protein profiling by mass spectrometry using label-free proteomics. Methods Mol Biol 2008;439:241–56.
27. Burlina F, Sagan S, Bolbach G, Chassaing G. A direct approach to quantification of the cellular uptake of cell-penetrating peptides using MALDI-TOF mass spectrometry. Nat Protoc 2006;1:200–5.
28. Gerber SA, Rush J, Stemman O, Kirschner MW, Gygi SP. Absolute quantification of proteins and phosphoproteins from cell lysates by tandem MS. Proc Natl Acad Sci U S A 2003;100:6940–5.
29. Pratt JM, Simpson DM, Doherty MK, Rivers J, Gaskell SJ, Beynon RJ. Multiplexed absolute quantification for proteomics using concatenated signature peptides encoded by QconCAT genes. Nat Protoc 2006;1:1029–43.
30. Rivers J, Simpson DM, Robertson DH, Gaskell SJ, Beynon RJ. Absolute multiplexed quantitative analysis of protein expression during muscle development using QconCAT. Mol Cell Proteomics 2007;6:1416–27.
31. Yocum AK, Chinnaiyan AM. Current affairs in quantitative targeted proteomics: multiple reaction monitoring-mass spectrometry. Brief Funct Genomic Proteomic 2009;8:145–57.
32. Kitteringham NR, Jenkins RE, Lane CS, Elliott VL, Park BK. Multiple reaction monitoring for quantitative biomarker analysis in proteomics and metabolomics. J Chromatogr B Analyt Technol Biomed Life Sci 2009;877:1229–39.
33. Kim K, Kim Y. Preparing multiple-reaction monitoring for quantitative clinical proteomics. Expert Rev Proteomics 2009;6:225–9.
34. Addona TA, Abbatiello SE, Schilling B, Skates SJ, Mani DR, Bunk DM, *et al*. Multi-site assessment of the precision and reproducibility of multiple reaction monitoring-based measurements of proteins in plasma. Nat Biotechnol 2009;27:633–41.
35. Anderson NL, Anderson NG, Haines LR, Hardie DB, Olafson RW, Pearson TW. Mass spectrometric quantitation of peptides and proteins using Stable Isotope Standards and Capture by Anti-Peptide Antibodies (SISCAPA). J Proteome Res 2004;3:235–44.
36. Petricoin 3rd EF, Hackett JL, Lesko LJ, Puri RK, Gutman SI, Chumakov K, *et al*. Medical applications of microarray technologies: a regulatory science perspective. Nat Genet 2002;32(Suppl.):474–9.
37. Cadieux PA, Beiko DT, Watterson JD, Burton JP, Howard JC, Knudsen BE, *et al*. Surface-enhanced laser desorption/ionization-time of flight-mass spectrometry (SELDI-TOF-MS): a new proteomic urinary test for patients with urolithiasis. J Clin Lab Anal 2004;18:170–5.
38. Seibert V, Wiesner A, Buschmann T, Meuer J. Surface-enhanced laser desorption ionization time-of-flight mass spectrometry (SELDI TOF-MS) and ProteinChip technology in proteomics research. Pathol Res Pract 2004;200:83–94.
39. Kricka LJ, Master SR. Validation and quality control of protein microarray-based analytical methods. Mol Biotechnol 2008;38:19–31.
40. Zhu H, Snyder M. Protein chip technology. Curr Opin Chem Biol 2003;7:55–63.
41. VanMeter A, Signore M, Pierobon M, Espina V, Liotta LA, Petricoin 3rd EF. Reverse-phase protein microarrays: application to biomarker discovery and translational medicine. Expert Rev Mol Diagn 2007;7:625–33.
42. Gillette MA, Mani DR, Carr SA. Place of pattern in proteomic biomarker discovery. J Proteome Res 2005;4:1143–54.
43. Zhang H, Liu AY, Loriaux P, Wollscheid B, Zhou Y, Watts JD, *et al*. Mass spectrometric detection of tissue proteins in plasma. Mol Cell Proteomics 2007;6:64–71.
44. Anderson NL, Polanski M, Pieper R, Gatlin T, Tirumalai RS, Conrads TP, *et al*. The human plasma proteome: a nonredundant list developed by combination of four separate sources. Mol Cell Proteomics 2004;3:311–26.
45. Nedelkov D, Kiernan UA, Niederkofler EE, Tubbs KA, Nelson RW. Investigating diversity in human plasma proteins. Proc Natl Acad Sci U S A 2005;102:10852–7.
46. Schuchard MD, Mehigh RJ, Cockrill SL, Lipscomb GT, Stephan JD, Wildsmith J, *et al*. Artifactual isoform profile modification following treatment of human plasma or serum with protease inhibitor, monitored by 2-dimensional electrophoresis and mass spectrometry. Biotechniques 2005;39:239–47.
47. Xiang Y, Sekine T, Nakamura H, Imajoh-Ohmi S, Fukuda H, Nishioka K, *et al*. Proteomic surveillance of autoimmunity in osteoarthritis: identification of triosephosphate isomerase as an autoantigen in patients with osteoarthritis. Arthritis Rheum 2004;50:1511–21.
48. Matsuo K, Xiang Y, Nakamura H, Masuko K, Yudoh K, Noyori K, *et al*. Identification of novel citrullinated autoantigens of synovium in rheumatoid arthritis using a proteomic approach. Arthritis Res Ther 2006;8:R175.
49. de Seny D, Fillet M, Meuwis MA, Geurts P, Lutteri L, Ribbens C, *et al*. Discovery of new rheumatoid arthritis biomarkers using

the surface-enhanced laser desorption/ionization time-of-flight mass spectrometry ProteinChip approach. Arthritis Rheum 2005;52:3801–12.
50. Schulz M, Dotzlaw H, Mikkat S, Eggert M, Neeck G. Proteomic analysis of peripheral blood mononuclear cells: selective protein processing observed in patients with rheumatoid arthritis. J Proteome Res 2007;6:3752–9.
51. Peirce MJ, Saklatvala J, Cope AP, Wait R. Mapping lymphocyte plasma membrane proteins: a proteomic approach. Methods Mol Med 2007;136:361–7.
52. Liao H, Wu J, Kuhn E, Chin W, Chang B, Jones M, *et al*. Use of mass spectrometry to identify protein biomarkers of disease severity in the synovial fluid and serum of patients with rheumatoid arthritis. Arthritis Rheum 2004;50:3792–803.
53. Yamagiwa H, Sarkar G, Charlesworth MC, McCormick DJ, Bolander ME. Two-dimensional gel electrophoresis of synovial fluid: method for detecting candidate protein markers for osteoarthritis. J Orthop Sci 2003;8:482–90.
54. Sinz A, Bantscheff M, Mikkat S, Ringel B, Drynda S, Kekow J, *et al*. Mass spectrometric proteome analyses of synovial fluids and plasmas from patients suffering from rheumatoid arthritis and comparison to reactive arthritis or osteoarthritis. Electrophoresis 2002;23:3445–56.
55. Drynda S, Ringel B, Kekow M, Kühne C, Drynda A, Glocker M, *et al*. Proteome analysis reveals disease-associated marker proteins to differentiate RA patients from other inflammatory joint diseases with the potential to monitor anti-TNFalpha therapy. Pathol Res Pract 2004;200:165–71.
56. Kantor A, Wang W, Lin H, Govindarajan H, Anderle M, Perrone A, *et al*. Biomarker discovery by comprehensive phenotyping for autoimmune diseases. Clin Immunol 2004;111:186–95.
57. Kuhn E, Wu J, Karl J, Liao H, Zolg W, Guild B. Quantification of C-reactive protein in the serum of patients with rheumatoid arthritis using multiple reaction monitoring mass spectrometry and ^{13}C-labeled peptide standards. Proteomics 2004;4:1175–86.
58. Gobezie R, Kho A, Krastins B, Sarracino DA, Thornhill TS, Chase M, *et al*. High abundance synovial fluid proteome: distinct profiles in health and osteoarthritis. Arthritis Res Ther 2007;9:R36.
59. Kamphorst J, van der Heijden R, DeGroot J, Lafeber F, Reijmers T, van El B, *et al*. Profiling of endogenous peptides in human synovial fluid by NanoLC–MS: method validation and peptide identification. J Proteome Res 2007;6:4388–96.
60. Uchida T, Fukawa A, Uchida M, Fujita K, Saito K. Application of a novel protein biochip technology for detection and identification of rheumatoid arthritis biomarkers in synovial fluid. J Proteome Res 2002;1:495–9.
61. Garnero P, Charni N, Juillet F, Conrozier T, Vignon E. Increased urinary type II collagen helical and C telopeptide levels are independently associated with a rapidly destructive hip osteoarthritis. Ann Rheum Dis 2006;65:1639–44.
62. Meulenbelt I, Kloppenburg M, Kroon HM, Houwing-Duistermaat JJ, Garnero P, Hellio Le Graverand MP, *et al*. Urinary CTX-II levels are associated with radiographic subtypes of osteoarthritis in hip, knee, hand, and facet joints in subject with familial osteoarthritis at multiple sites: the GARP study. Ann Rheum Dis 2006;65:360–5.
63. Hellio Le Graverand MP, Brandt KD, Mazzuca SA, Katz BP, Buck R, Lane KA, *et al*. Association between concentrations of urinary type II collagen neoepitope (uTIINE) and joint space narrowing in patients with knee osteoarthritis. Osteoarthritis Cartilage 2006;14:1189–95.
64. Barratt J, Topham P. Urine proteomics: the present and future of measuring urinary protein components in disease. CMAJ 2007;177:361–8.
65. Li WW, Nemirovskiy O, Fountain S, Rodney Mathews W, Szekely-Klepser G. Clinical validation of an immunoaffinity LC–MS/MS assay for the quantification of a collagen type II neoepitope peptide: a biomarker of matrix metalloproteinase activity and osteoarthritis in human urine. Anal Biochem 2007;369:41–53.
66. Nemirovskiy OV, Dufield DR, Sunyer T, Aggarwal P, Welsch DJ, Mathews WR. Discovery and development of a type II collagen neoepitope (TIINE) biomarker for matrix metalloproteinase activity: from in vitro to in vivo. Anal Biochem 2007;361:93–101.
67. Lamers RJ, van Nesselrooij JH, Kraus VB, Jordan JM, Renner JB, Dragomir AD, *et al*. Identification of an urinary metabolite profile associated with osteoarthritis. Osteoarthritis Cartilage 2005;13:762–8.
68. Hermansson M, Sawaji Y, Bolton M, Alexander S, Wallace A, Begum S, *et al*. Proteomic analysis of articular cartilage shows increased type II collagen synthesis in osteoarthritis and expression of inhibin betaA (activin A), a regulatory molecule for chondrocytes. J Biol Chem 2004;279:43514–21.
69. De Ceuninck F, Marcheteau E, Berger S, Caliez A, Dumont V, Raes M, *et al*. Assessment of some tools for the characterization of the human osteoarthritic cartilage proteome. J Biomol Tech 2005;16:256–65.
70. Stevens AL, Wishnok JS, Chai DH, Grodzinsky AJ, Tannenbaum SR. A sodium dodecyl sulfate-polyacrylamide gel electrophoresis-liquid chromatography tandem mass spectrometry analysis of bovine cartilage tissue response to mechanical compression injury and the inflammatory cytokines tumor necrosis factor alpha and interleukin-1 beta. Arthritis Rheum 2008;58:489–500.
71. Wilson R, Belluoccio D, Little CB, Fosang AJ, Bateman JF. Proteomic characterization of mouse cartilage degradation in vitro. Arthritis Rheum 2008;58:3120–31.
72. De Ceuninck F, Dassencourt L, Anract P. The inflammatory side of human chondrocytes unveiled by antibody microarrays. Biochem Biophys Res Commun 2004;323:960–9.
73. Catterall JB, Rowan AD, Sarsfield S, Saklatvala J, Wait R, Cawston TE. 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 (Oxford) 2006;45:1101–9.
74. Haglund L, Bernier SM, Onnerfjord P, Recklies AD. Proteomic analysis of the LPS-induced stress response in rat chondrocytes reveals induction of innate immune response components in articular cartilage. Matrix Biol 2008;27:107–18.
75. Azad NS, Rasool N, Annunziata CM, Minasian L, Whiteley G, Kohn EC. Proteomics in clinical trials and practice: present uses and future promise. Mol Cell Proteomics 2006;5:1819–29.
76. Cornett DS, Reyzer ML, Chaurand P, Caprioli RM. MALDI imaging mass spectrometry: molecular snapshots of biochemical systems. Nat Methods 2007;4:828–33.
77. Andersson M, Groseclose MR, Deutch AY, Caprioli RM. Imaging mass spectrometry of proteins and peptides: 3D volume reconstruction. Nat Methods 2008;5:101–8.
78. Lemaire R, Desmons A, Tabet JC, Day R, Salzet M, Fournier I. Direct analysis and MALDI imaging of formalin-fixed, paraffin-embedded tissue sections. J Proteome Res 2007;6:1295–305.
79. Aigner T, Haag J, Zimmer R. Functional genomics, evo-devo and systems biology: a chance to overcome complexity? Curr Opin Rheumatol 2007;19:463–70.
80. Bauer DC, Hunter DJ, Abramson SB, Attur M, Corr M, Felson D, *et al*. Classification of osteoarthritis biomarkers: a proposed approach. Osteoarthritis Cartilage 2006;14:723–7.