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Assessment of Some Tools for the Characterization of the Human Osteoarthritic Cartilage Proteome

Frédéric De Ceuninck,[1] Estelle Marcheteau,[2] Sylvie Berger,[2] Audrey Caliez,[1] Valérie Dumont,[3] Martine Raes,[3] Philippe Anract,[4] Grégory Leclerc,[2] Jean A Boutin,[2] and Gilles Ferry[2]

[1]Institut de Recherches Servier, Division de Rhumatologie, Suresnes, France; [2]Institut de Recherches Servier, Pharmacologie Moléculaire et Cellulaire, Croissy-sur-Seine, France; [3]Université de Namur, Namur, Belgium: [4]Hôpital Cochin, Orthopédie B, Paris, France

Since the proteome of osteoarthritic articular cartilage has been poorly investigated as yet, we adapted proteomic technologies to the study of the proteins secreted or released by fresh human osteoarthritic cartilage in culture. Fresh cartilage explants were obtained from three donors undergoing surgery for knee joint replacement. The explants were dissected out, minced, and incubated in serum-free culture medium. After 48 h, proteins in the medium were identified by two-dimensional or off-gel electrophoresis coupled to tandem mass spectrometry, or by using an antibody-based protein microarray designed to detect angiogenic factors, growth factors, chemokines, and cytokines. We identified a series of 43 proteins. Some of these proteins were already described as secretion products of chondrocytes, such as YKL-39 or osteoprotegerin, while several other were known proteins but have never been reported previously in cartilage, such as the serum amyloid P-component, the vitamin D binding protein, the pigment epithelium derived factor, the pulmonary and activation-regulated chemokine, lyl-1, thrombopoietin, fibrinogen, angiogenin, gelsolin, and osteoglycin/mimecan. While this study enabled the identification of novel proteins secreted or released by human osteoarthritic cartilage, the goal of the present work was essentially to describe the technical approach necessary for a systematic study of osteoarthritic cartilages from a large population of donors, in order to be able to select the good markers and/or targets for this poorly explored disease.

Key Words: Cartilage; osteoarthritis; proteome; protein; identification.

Address correspondence and reprint requests to: F. De Ceuninck, PhD, Institut de Recherches Servier, Division de Rhumatologie, 11 rue des Moulineaux, 92150 Suresnes, France (phone: + 33–1-5572–2723; fax: + 33–1-5572–2740; email: frederic.deceuninck@fr.netgrs.com).

Over the last decade, the awareness that proteins are the main effectors of biological responses, and most often the causative agents of diseases, has expanded the field of proteomics. The word "proteome" appeared in 1996 to define proteins expressed by the genome of a tissue or of differentiated cells.[1] Since then, a wide arsenal of techniques has been used to identify proteins expressed by tissues or cells, or to compare differential protein expression in healthy and diseased states. To date, two-dimensional polyacrylamide gel electrophoresis (2D-PAGE),[2–5] associated with protein identification by mass spectrometry (MS)[6,7] and bioinformatics[8] remains the most widely used technique. A number of 2D-PAGE maps depicting proteins spots on 2D-gels and their identification can be found in databases accessible on the internet.[9] These resources are inestimable contributions to researchers working on the corresponding tissues or cell types, and they can be fully integrated in projects for a better understanding of diseases mechanisms. Apart from "conventional" 2D-PAGE, some emerging technologies, such as isotope-coded affinity tag, liquid chromatography coupled with tandem MS, off-gel electrophoresis, and protein chips or protein microarrays, also represent powerful tools for the identification of proteins.[10–14]

Over the last few years, proteomics has increased the understanding of a large number of diseases.[15–21]

However, there is a striking lack of such knowledge in rheumatology. Only a few genomic approaches were used to compare gene expression in fetal, normal, or osteoarthritic (OA) chondrocytes by analyses of expressed sequenced tags or cDNA-array.[22–25] The analysis of protein secretion by human chondrocytes or cartilage using 2D-PAGE or other proteomic technologies has not yet been performed, although 2D-gel electrophoresis was used to identify proteins synthesized by fetal bovine chondrocytes.[26] More recently, proteomic approaches were developed to identify candidate protein markers in the synovial fluid or synovium of patients with OA or rheumatoid arthritis (RA).[27–30] However, there is no report on such protein profiling of pathological cartilage.

In the present study, three different technologies, 2D- and off-gel electrophoresis coupled to MS, and antibody-based microarray were used to identify proteins secreted or released by human OA cartilage. The present pilot study aimed at the description and characterization of the tools necessary to conduct a larger study based on numerous samples. It essentially shows the possibility of using the currently described methodology to identify markers or targets of osteoarthritis.

MATERIALS AND METHODS

Cartilage Samples

Osteoarthritic articular cartilages were recovered from femoral condyles and tibial plateaus of three patients undergoing surgical intervention for knee joint replacement. Donors 1, 2, and 3 were a 54-yr-old female, an 80-yr-old male, and a 53-yr-old female, respectively. After the operation, cartilage still attached to bony pieces was put in serum-free Ham F12 medium and conveyed to the laboratory in less than 2 h. The cartilage specimen were chosen among others for proteomic analysis because of the relative integrity of the remaining articular cartilage areas. Upon receipt, cartilage slices were carefully dissected out so as to avoid bony, bloody, or fibrous pieces. Cartilage explants were washed extensively in serum-free Ham F12 medium for 2 h with three renewals. Then, explants were dispensed in a 100-mm diameter Petri dish in 40 mL of Ham F12 medium without any additive and incubated at 37°C under 5% CO_2 for 48 h.

Harvest and Conditioning of Secretion Medium

All steps were performed at 4°C. The secretion medium was harvested and immediately supplemented with a protease inhibitor cocktail covering a broad spectrum of proteases (Complete; Roche, Meylan, France). The medium was cleared by centrifugation at 3400 × g for 15 min. Two-dimensional electrophoresis cannot be achieved easily when salts or highly anionic compounds are present in the medium, since the first separation dimension rests on the net charge of proteins. To remove high-molecular- mass anionic proteoglycans, the medium was filtered in a device fitted with a 100-kDa cut-off filter membrane (Amicon, Beverly, MA). The filtrate (Mr < 100 kDa) was filtered on a 10-kDa cut-off filter membrane at 4°C to remove salts and concentrate the sample. The retentate (10 kDa < Mr < 100 kDa) was kept for proteomic analyses.

One- and Two-Dimensional Gel Electrophoresis of Secreted Proteins

Proteins were solubilized in 7 M urea, 2 M thiourea, 4% CHAPS, 20 mM dithiothreitol (DTT), 2 mM tributyl phosphine, and 0.5% immobilized pH gradient (IPG) buffer (67% 4–7 and 33% 6–8 (v/v). The mixture was centrifuged at 100,000 × g for 1 h and the supernatant was transferred to a fresh tube for 2D gel electrophoresis. The pellet containing hydrophobic proteins was solubilized in Laemmli buffer for 1D gel electrophoresis. The first dimension of 2D gel electrophoresis was performed on a IPGphor isoelectric focusing unit (Amersham Biosciences, Buckinghamshire, UK) using ReadyStrip Immobilized pH gradient (IPG) strips (pH range 4–7, 170 × 3 × 0.5 mm, BioRad (Hercules, CA) according to the manufacturer's instructions. Briefly, the IPG strips were rehydrated passively with 100 µg of sample for 7 h, followed by 1 h of active rehydration (20°C, 30 V). The total voltage-hour during isoelectrofocalization was 32 kVh. After isoelectrofocalization, strips were equilibrated twice for 20 min in 6 M urea, 2% sodium dodecylsulfate (SDS), 0.375 M Tris (pH 8.8), 20% glycerol, with 130 mM DTT and 135 mM iodoacetamide, successively. The second dimension was performed on a 10% SDS-polyacrylamide gel electrophoresis (PAGE) Protean II ready gel (BioRad). The protein spots were visualized by Sypro ruby staining (Biorad) using a Typhoon 9400 (Amersham Biosciences) fluorescence gel scanner. Qualitative spot detection was performed with the PDQUEST software (Biorad).

Off-Gel Electrophoresis

Off-gel electrophoresis was performed in a minirotofor apparatus (Biorad). The sample (1.12 mg/700 µL) was put in the minirotofor chamber previously subjected to a voltage of 300 V for 1 h with 19 mL of equilibration

buffer, so as to obtain the pH gradient. Focusing was performed in three steps of 1 h each, at 500 V, 800 V, and 1000 V, successively, by supplying a power of 12 W and an intensity of 25 mA. The 20 focused fractions were collected at the bottom of chambers via 20 needles connected to a vacuum source. Proteins in each fraction were precipitated (2D-Clean-Up kit, Amersham Biosciences) and resolubilized with Laemmli buffer prior to 1D SDS-PAGE.

Excision of Proteins and Identification by Tandem MS

Spots were excised from gels manually, and transferred to 1.5 mL siliconized Eppendorf tubes. One protein-free gel piece was treated in parallel as a negative control. Washing steps and digestions were realized according to Shevchenko et al.[31] Gel pieces were washed with 50 mM ammonium bicarbonate buffer (pH 7.9) and dehydrated with pure acetonitrile. After drying in a Speedvac (Heto, Denmark) they were incubated for 45 min at 56°C in ammonium bicarbonate buffer (pH 7.9) containing 10 mM DTT and alkylated at 22°C in a dark room for 30 min with 55 mM iodoacetamide. After successive washing steps, gel pieces were completely dried in the speed-vac. Proteins in gels were subjected to trypsin digestion (Promega, Madison, WI) overnight at 37°C. Peptide digests were extracted successively with pure acetonitrile and 5% formic acid, and dried. They were diluted in 5% formic acid and loaded onto a geloader pipette tip C18 resin (Proxeon, Odense, Denmark), then eluted with 0.8 µL of 50% methanol 0.1% formic acid (v/v) directly into nanospray capillary needles (Waters, Milford, MA). Peptides were analyzed on a quadrupole time-of-flight (qTOF) 2 mass spectrometer (Waters). MS acquisitions were performed within the mass range of 550 to 1300 *m/z* and MS/MS within 50 and 2000 *m/z*.

Database Searching and Sequence Analysis

SWISS-PROT and TREMBL with BLAST interface (US National Center for Biotechnology Information) were used to identify proteins on the basis of amino acid sequences. Partial amino acid sequences (sequence TAG) were used to identify matches in databases with PeptideSearch interface (EMBL, Heidelberg, Germany). Matches were confirmed by performing a theoretical trypsin digestion with GPMAW (Lighthouse data, Denmark) and comparing the resulting peptide mass and sequence to those obtained experimentally.

Protein Microarrays

Antibody microarray (RayBio Human Cytokine Arrays, RayBiotech, Inc.) experiments were performed as recommended by the manufacturer. Briefly, array membranes were blocked for 30 min and incubated for 90 min with 1 mL of crude conditioned medium. Then, biotinylated antibodies were added on membranes for 45 min. Detection was achieved with peroxidase-labeled streptavidin followed by chemiluminescence and capture of the signals on x-ray films.

RESULTS

Identification of Proteins Secreted by Human OA Cartilage of Donor 1 by 1D- and 2D-Gel Electrophoresis, and Off-Gel Electrophoresis

Two-dimensional gel electrophoresis of the incubation medium of cartilage explants is a tricky operation, since the culture medium by itself contains high amounts of salts, and the proteins secreted include a variety of highly anionic compounds, all of which complicate the first dimension analysis (isoelectrofocalization). Even with a careful prefractionation of the secretion medium, the persistence of some of these compounds inevitably led to a certain extent of horizontal streaking (Fig. 1). To complicate matters, cartilage explants secreted a few abundant proteins that may hide the less abundant ones. This matter cannot be easily circumvented in 2D gel analyses, since the amount of proteins to be loaded in the first dimension is limited. Twenty-nine individualized spots were excised, destained, and analyzed by MS. In some cases, the same protein was identified for distinct spots, reflecting posttranslational modifications, and inversely, several proteins were found under one single spot, reflecting similar Mr/pI properties. In some cases, protein identification was not possible because no match or more than one match was found in databases. Finally, some proteins were in amounts too low to produce exploitable results by MS. The proteins identified are indicated in Table 1 (spot numbers 1–29).

Off-gel electrophoresis using the minirotofor apparatus provides a new mean of discarding abundant proteins and of concentrating the less abundant ones. Seventeen of the twenty focused fractions (pH range 2.9–10.4) led to visible amounts of proteins after 1D SDS-PAGE (Fig. 2). Nineteen spots were excised and analysed by MS. The proteins identified are indicated in Table 1 (spot numbers 30–48).

Finally, hydrophobic proteins (i.e., proteins present in the pellet after centrifugation of the conditioned

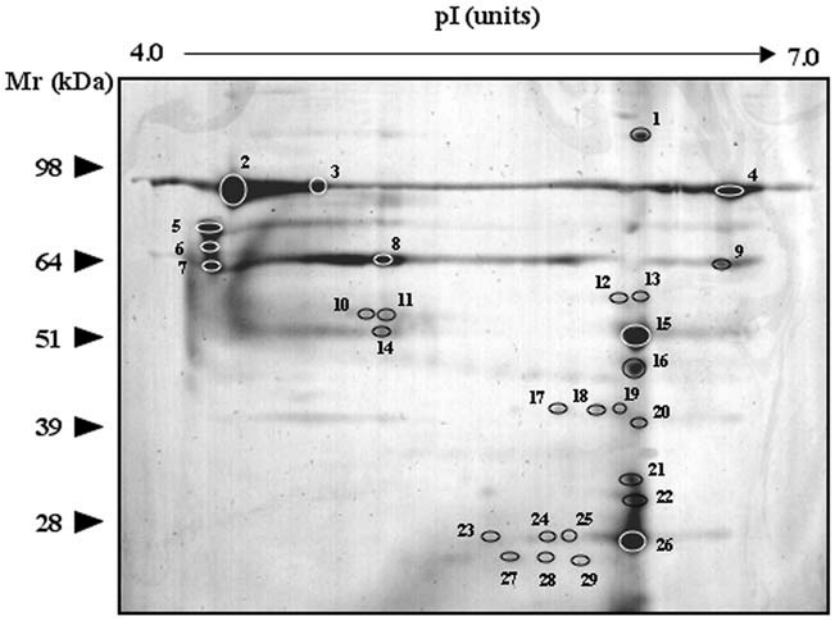


FIGURE 1

Two-dimensional electrophoretic map of the conditioned secretion medium of osteoarthritic cartilage explants coming from donor 1. Proteins were stained by Sypro ruby. *Circled* and *numbered spots* were excised for further identification by mass spectrometry.

TABLE 1

Proteins Identified in the Secretion Medium of Cartilage from Donor 1

Spot No.	Accession No.	Protein Product
2,20	P12980	Lyl-1 protein
2	NP_009043	Thrombospondin 3 (TSP-3)
2–6,30,31,50,51	BAC53888	Cartilage oligomeric matrix protein (COMP)
8,32,42,53	CAA23754	Serum albumin
15	P01859	Immunoglobulin gamma-2 chain C region
22	AAH00866	Tissue inhibitor of metalloproteinase-1 (TIMP-1)
26	AAA73002	Human immunoglobulin rearranged gamma chain
33	AAA36321	Matrix metalloproteinase-3 (MMP-3, stromelysin-1)
34	CAA27173	Unnamed protein product
34	AAA84914	Pigment epithelium-derived factor (PEDF)
34,54	NP_002336	Lumican
35	P02675	Fibrinogen β-chain
36	AAA51765	Apolipoprotein J (clusterin)
37	NP_054776	Osteoglycin (osteoinductive factor, mimecan)
38	P02743	Serum amyloid P-component
38	NP_001056	TNFα receptor 1, (TNFR1, TNF-R55)
40	P20305	Gelsolin (actin-depolymerizing factor, ADF)
44	AAB24608	IgG1 heavy chain
45	NP_003991	YKL-39 (chitinase 3-like 2 protein)
48	1GFKA	Mutant human lysozyme
49,50	CAA26536	Fibronectin
52	AAH00163	Vimentin
57	AAC17968	Immunoglobulin kappa

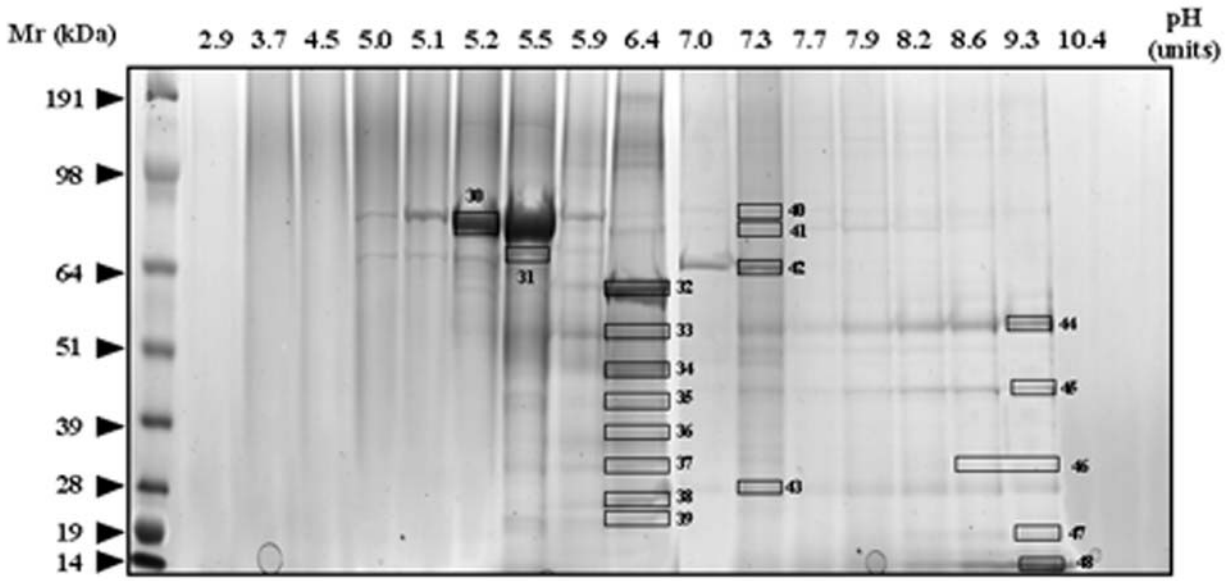


FIGURE 2

Off-gel electrophoresis of the conditioned secretion medium of OA cartilage explants coming from donor 1. Seventeen fractions collected after Off-gel electrophoresis were precipitated by the clean-up procedure (Amersham Biosciences) and subjected to SDS-PAGE. Proteins were stained by Sypro ruby. *Framed, numbered bands* were excised for further identification by mass spectrometry.

culture medium solubilized in urea/thiourea buffer) were separated by 1D-SDS-PAGE (Fig. 3). Analysis by MS enabled the identification of 3 peptides (spot numbers 49, 50, 52, 57).

Using these three techniques, 22 proteins were identified from the cartilage of donor 1.

Identification of Proteins Secreted by Human OA Cartilage of Donor 2 by 2D Gel Electrophoresis

Two-dimensional gel analysis was performed from the incubation medium of cartilage explants coming from donor 2. The experiment was reproduced twice. The electrophoretic maps are shown in Figure 4. Forty-six spots were excised and analyzed by MS. Nineteen spots yielded unambiguous identification of fourteen proteins, summarized in Table 2. Lumican, serum amyloid P-component, immunoglobulin G (IgG)-2 chain C, albumin, and fibrinogen were found to be in common with proteins identified in donor 1.

Analysis of Proteins Secreted by Human OA Cartilage of Donor 3 by Antibody Microarrays

Electrophoretic techniques are limited for low-molecular-weight proteins as well as for minor or poorly stained proteins. These proteins may remain hidden despite their potential functional importance. Because

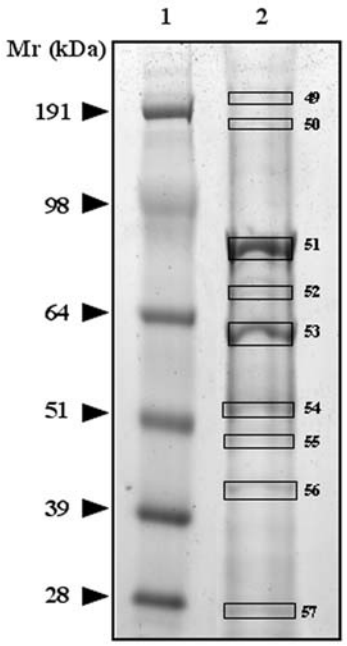


FIGURE 3

Sodium dodecylsulfate-polyacrylamide gel electrophoresis of the nonsoluble fraction of the conditioned secretion medium of osteoarthritic cartilage explants coming from donor 1. Proteins were stained by Sypro ruby. *Framed, numbered bands* were excised for further identification by mass spectrometry.

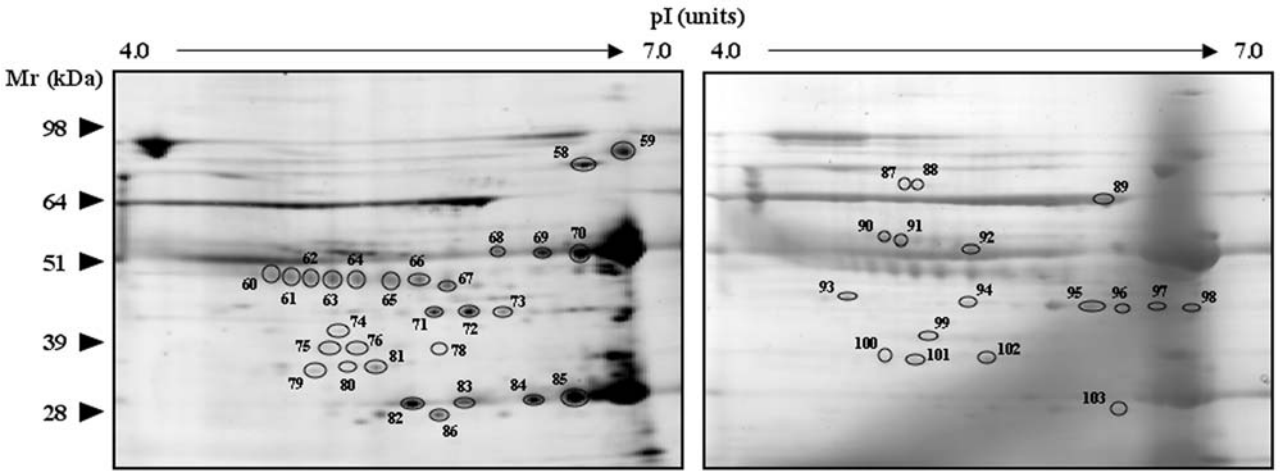


FIGURE 4

Two-dimensional electrophoretic maps of the conditioned secretion medium of osteoarthritic cartilage explants coming from donor 2. Two maps resulting from two different analyses with the same conditioned medium are shown. Proteins were stained by Sypro ruby. *Circled* and *numbered* spots were excised for futher identification by mass spectrometry.

of their sensitivity, protein microarrays help bypassing this problem. The patterns of two antibody arrays of proteins in the culture medium of cartilage explants from donor 3 are shown in Figure 5. Eleven proteins were identified and are symbolized by the red color in the comprehensive array tables.

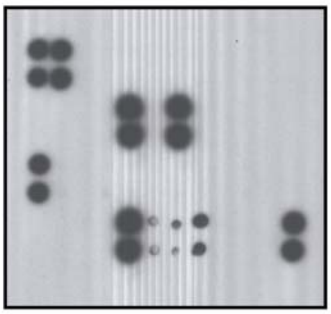
DISCUSSION

The catalyst for launching this project was that OA still remains a misunderstood pathology, despite its increasing prevalence in developed countries. The protein profile of different diseased tissues has been

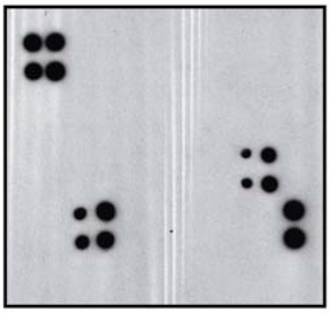
TABLE 2

Proteins Identified in theSecretion Medium of Cartilage from Donor 2

Spot No.	Accession No.	Protein Product
60,61	P51884	Lumican
82	P02743	Serum amyloid P-component
83-85	P01859	Immunoglobulin gamma-2 chain C region
87	NP_004521	Matrix metalloproteinase-2 (MMP-2, gelatinase A)
89	CAA23754	Serum albumin
90,91	1KCT	α1-Antitrypsin
92	1KW2A	Vitamin D-binding protein
95	P02675	Fibrinogen β-chain
97	CAA39666	Complement factor H-related protein 1 (FHR-1)
101	Q15113	Type I procollagen C-proteinase enhancer (PCPE)
103	NP_003269	Tetranectin (plasminogen-binding protein)
75,76	AAP88927	Apolipoprotein J (clusterin)
81	Q8EWH5	Glycerol-3-phosphate dehydrogenase
78	Q24451	α-Mannosidase II



	1	2	3	4	5	6	7	8	9	10	11	12
A	Pos	Pos	Neg	Neg	ENA-78	GCSF	GM-CSF	GRO	GRO-α	I-309	IL-1α	IL-1β
B	Pos	Pos	Neg	Neg	ENA-78	GCSF	GM-CSF	GRO	GRO-α	I-309	IL-1α	IL-1β
C	IL-2	IL-3	IL-4	IL-5	IL-6	IL-7	IL-8	IL-10	IL-12	IL-13	IL-15	IFN-γ
D	IL-2	IL-3	IL-4	IL-5	IL-6	IL-7	IL-8	IL-10	IL-12	IL-13	IL-15	IFN-γ
E	MCP-1	MCP-2	MCP-3	MCSF	MDC	MIG	MIP-1δ	RANTES	SCF	SDF-1	TARC	TGF-β1
F	MCP-1	MCP-2	MCP-3	MCSF	MDC	MIG	MIP-1δ	RANTES	SCF	SDF-1	TARC	TGF-β1
G	TNF-α	TNF-β	EGF	IGF-I	Ang	OSM	Tpo	VEGF	PDGF B	Leptin	Neg	Pos
H	TNF-α	TNF-β	EGF	IGF-I	Ang	OSM	Tpo	VEGF	PDGF B	Leptin	Neg	Pos



	1	2	3	4	5	6	7	8	9	10	11	12
A	Pos	Pos	Neg	Neg	BDNF	BLC	Ckβ 8-1	Eotaxin	Eotaxin-2	Eotaxin-3	FGF-4	FGF-6
B	Pos	Pos	Neg	Neg	BDNF	BLC	Ckβ 8-1	Eotaxin	Eotaxin-2	Eotaxin-3	FGF-4	FGF-6
C	FGF-7	FGF-9	Fit-3 Ligand	Fractalkine	GCP-2	GDNF	HGF	IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4	IL-16
D	FGF-7	FGF-9	Fit-3 Ligand	Fractalkine	GCP-2	GDNF	HGF	IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4	IL-16
E	IP-10	LIF	LIGHT	MCP-4	MIF	MIP-3α	NAP-2	NT-3	NT-4	OPG	PARC	PIGF
F	IP-10	LIF	LIGHT	MCP-4	MIF	MIP-3α	NAP-2	NT-3	NT-4	OPG	PARC	PIGF
G	TGF-β 2	TGF-β 3	TIMP-1	TIMP-2	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Pos
H	TGF-β 2	TGF-β 3	TIMP-1	TIMP-2	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Pos

FIGURE 5

Protein microarrays (A and B) of proteins secreted in the secretion medium of OA cartilage explants coming from donor 3. The comprehensive maps of these arrays are shown, with positive hits on the arrays appearing in *red*.

shown to implement substantially the understanding of pathobiological processes, by the identification of new biological targets. Although the present study does not claim to give an exhaustive and complete profile of proteins secreted by human OA cartilage (which is a pharaonic and technically tricky job, if ever possible) it enabled the identification of several proteins, many of which had not been previously reported in cartilage.

Two-dimensional gel electrophoresis coupled with MS is still the method of choice for protein profiling. Nevertheless, several complications were encountered during experiments with the secretion medium of cartilage. Abundant anionic compounds and the high amount of salts disturbed migration during the first dimension, and even after careful filtration steps to discard high-molecular-weight molecules (proteoglycans) and low-molecular-weight com-

pounds (salts), horizontal streaks remained on gels. Furthermore, the amount of cartilage harvested from OA donors was relatively poor. Thus, the amount of proteins present in the incubation medium was relatively low and prevented too many prefractionation steps, which should have led to sample loss. Additionally, the overall proteome was represented by a low number of abundant proteins, exemplified by cartilage oligomeric matrix protein. Since in the 2D approach, only a limited amount of proteins can be loaded onto a gel, the less-abundant proteins could hardly be detected on stained 2D gels. Off-gel electrophoresis helped bypass this problem, since a higher amount of proteins could be analyzed, thus enabling the identification of less-abundant products. Additionally, antibody microarrays implemented these two technologies by targeting chemokines, cytokines, and growth factors. Some important considerations should be kept in mind before discussing the relevance of proteins identified in the present study. First, spots on gels may result from posttranslational modifications, and distinct spots may reflect a single protein. Secondly, it is possible that some of the proteins released from cartilage into the medium may have come into cartilage from synovial fluid or subchondral bone. Thus, proteins in the medium may be secretion or diffusion products. Hypotheses on their function and their potential relevance in cartilage pathobiology are rapidly reviewed below.

The first striking finding was that some of the peptides identified by MS matched with various Ig chains and a member of the complement and immune regulators factor H family, FHR-1[32]. These proteins may have originated from the synovial fluid, raising the possibility that OA cartilages were surrounded by components of the immune response, since these samples came from end-stage OA donors, where immune reactions may be more developed than in early OA. Another unexpected finding was the identification of peptides matching with lyl-1[33], fibrinogen, and the serum amyloid P-component. The acute-phase reactant protein fibrinogen was found to be secreted by cartilage coming from both OA donors. Although it is sometimes increased in RA,[34] its secretion by cartilage has not been described. Serum amyloid P-component (SAP) is a protein named for its ubiquitous presence in amyloid deposits. Amyloidosis has been sporadically reported in juvenile rheumatoid arthritis[35,36] and could be involved in rheumatic diseases.[37] Strikingly, SAP was found in the two OA samples together with clusterin.[38] Similarly to observations in Alzheimer's disease,[39] clusterin may counteract some level of deleterious amyloidosis in OA. On the other hand, SAP may participate in the handling of chromatin exposed to cell death,[40] known as a crucial event in OA.

Of the other proteins identified, some are known as metabolic regulators of the cell, and their finding in the secretion medium of OA cartilage may be interpreted as an attempt to counteract catabolic processes [tissue inhibitor of metalloproteinase-1 (TIMP-1) and -2, pigment epithelium-derived factor (PEDF), α1-antitrypsin, tumor necrosis factor receptor 1 (TNFR1), osteoprotegerin, YKL-39]. PEDF was suggested to be a potential antiangiogenic factor in epiphyseal cartilage,[41] and it may have a similar role in articular cartilage in OA. Alpha1-antitrypsin belongs to the serine proteinase inhibitor (SERPIN) gene family and it may protect cartilage against degradation by inhibiting elastase activity.[42] Similarly, the secreted soluble form of TNF receptor 1 may reduce cartilage degradation by antagonizing the deleterious role of TNF on chondrocytes.[43,44] Also, acting as a decoy receptor for receptor activator of the nuclear factor κB ligand (RANKL, of which the membrane receptor RANK is a member of the TNF receptor family), osteoprotegerin may be an important target for pharmacological intervention in arthritis.[45,46] Finally, YKL-39, a member of the chitinase 18 family of proteins, may, as does its relative YKL-40, display anabolic properties in cartilage.[47,48]

On the other hand, some proteins may worsen the catabolic and/or proangiogenic pathways occuring in cartilage during OA such as matrix metalloproteinase (MMP)-2 and -3, vascular endothelial growth factor, osteoglycin, gelsolin, type I procollagen C-proteinase enhancer (PCPE), angiogenin, pulmonary and activation regulated chemokone (PARC), and thrombopoietin. Osteoglycin (osteoinductive factor),[49] may induce the mineralization and calcification of cartilage. Gelsolin is a depolymerizing factor of actin that mediates the reorganization of actin in articular chondrocytes after a hypoosmotic stress.[50,51] PCPE is a glycoprotein that binds the COOH-terminal propeptide of type I procollagen and potentiates its cleavage by procollagen C-proteinases,[52] potentially participating to the degenerative process. Neovascularization is also a hallmark of OA progression. As for vascular endothelial growth factor, cartilage-derived angiogenin may favor angiogenesis in OA. The function of cartilage-derived thrombopoietin remains more obscure, although it has been described as participating in the pathological thrombopoiesis of RA.[53]

Some further work has to be done to improve our knowledge of OA using proteomic technologies. Proteins identified in this study are only the visible part of the iceberg. Further investigations are also needed to confirm the relevance of these proteins in OA by performing comparative studies between OA and healthy donors. Nevertheless, the present study brings out potential novel important candidates for the treatment of OA.

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