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

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We show proteomic analysis can be applied to study cartilage pathophysiology. Proteins secreted by articular cartilage were analysed by 2-dimensional (2D) SDS-PAGE and mass spectrometry. Cartilage explants were cultured in medium containing \[^{35}\text{S}]\text{methionine/cysteine} to radiolabel newly synthesised proteins. In order to resolve the cartilage proteins by 2D-electrophoresis it was necessary to remove the proteoglycan aggrecan by precipitation with cetylpyridinium chloride.

50-100 radiolabelled protein spots were detected on 2D-gels of human cartilage cultures. Of 170 silver stained proteins identified, nineteen were radiolabelled representing newly synthesised gene products. Most of these were known cartilage constituents. Several non-radiolabelled cartilage proteins were also detected. The secreted protein pattern of explants from 12 osteoarthritic joints (knee, hip and shoulder) and 14 non-osteoarthritic adult joints were compared.

Synthesis of type II collagen was strongly up-regulated in osteoarthritic cartilage. Normal adult cartilage synthesised little or no type II collagen in contrast to infant and juvenile cartilage. Potential regulatory molecules novel to cartilage were identified: pro- and processed inhibin \(\beta\text{A} \) (which dimerises to activin A) were produced by all the osteoarthritic samples and half the normals. Connective tissue growth factor and cytokine-like protein C17 (previously only identified as an mRNA) were also found. Activin induced the tissue inhibitor for metalloproteinases-1 in human chondrocytes. Its expression was induced in isolated chondrocytes by growth factors or interleukin-1.

We conclude type II collagen synthesis in articular cartilage is down regulated at skeletal maturity and reactivated in osteoarthritis in attempted repair, and that activin A may be an anabolic factor in cartilage.
Osteoarthritis (OA) is a common joint disease characterised by degeneration of articular cartilage. Since cartilage has very limited capacity for repair the loss is effectively irreversible. Prevalence studies show that most people over the age of 65 have some evidence of the disease (1,2). Little is known about the molecular mechanism of cartilage destruction in OA, particularly the early events. It is thought that there is an imbalance between anabolism and catabolism of the extracellular matrix, there being an increase in catabolism. It has been suggested that this increased breakdown of matrix is due to production of degradative enzymes such as the matrix metalloproteinases (MMPs), and members of the disintegrin and metalloproteinase (ADAM) family (3,4). The increase in proteinase expression may be due to inflammatory cytokines such as interleukin-1 (IL-1) and tumour necrosis factor (4,5). However it is unclear whether these degradative processes are a primary event, or a secondary reaction.

Articular cartilage consists mainly of extracellular matrix whose principal organic components are type II collagen fibres and aggregates of the large proteoglycan aggrecan. The only cells in cartilage, the chondrocytes, contribute less than 5% to the total volume (6) and are responsible for the synthesis and degradation of matrix components. Very little is known about the normal endogenous control mechanisms of matrix turnover in articular cartilage. In order to study the regulation of synthesis of proteins in cartilage and to understand better the molecular basis of the osteoarthritic process we have developed a method for proteomic analysis of explanted tissue in which secreted proteins are separated using two-dimensional (2D) electrophoresis and identified by mass spectrometry (MS). We analysed secreted proteins because the tissue is mostly composed of extracellular material, and used metabolic radiolabelling to detect newly synthesised molecules. Limiting the study to secreted proteins means that only a few hundred need to be separated, which is feasible by
medium-format 2D gel electrophoresis. Since cartilage cannot be directly studied in the joint we used explanted human articular cartilage in culture.

Transcriptional analyses of normal and diseased cartilage have enabled parallel analysis of a large complement of genes in the osteoarthritic process (7,8). However mRNA levels do not necessarily correlate with protein expression and reveal nothing about processing or post-translational modification. A proteomic approach, in which proteins are identified and quantified directly, is therefore a valuable complement to such transcriptomic studies.

Our proteomic analysis shows a marked increase of type II collagen synthesis in osteoarthritic cartilage and has revealed that articular cartilage makes two potentially regulatory molecules, activin A and connective tissue growth factor (CTGF).
Materials and Methods

Materials.

$[^{35}S]$methionine/$[^{35}S]$cysteine and recombinant platelet-derived growth factor (PDGF) were purchased from Amersham Biosciences. Recombinant activin A and epidermal growth factor (EGF) were from R&D Systems and recombinant basic fibroblast growth factor (FGF-2) was obtained from PeproTech (London). Recombinant human IL-1α was prepared in house. The activin A ELISA kit came from R&D Systems, Abingdon, UK. Pronase E was from BDH. DMEM and FCS were obtained from BioWhittaker (Verviers, Belgium). All other reagents were the best available grade from Sigma.

Sources of cartilage.

Porcine articular cartilage was dissected from the metacarpophalangeal joints of 3-6 month old pigs within 24 h of slaughter. Human articular cartilage was obtained from Charing Cross Hospital, Hammersmith, London and the Royal National Orthopaedic Hospital, Stanmore, London with approval of the appropriate local ethical committee. Informed consent was given in all cases. OA cartilage came from joint replacement operations, while control samples were from femoral heads removed following trauma or from individuals undergoing amputations and resections for reasons other than joint disease. Some normal cartilage specimens were from fresh post mortem autopsies performed at Algemeen Ziekenhuis Sint-Jan, Bruges, Belgium or at the Department of Rheumatology, University of Ghent, Belgium. The age range was 22 to 86 years and 53 to 83 years for normal and osteoarthritic patients respectively. Details of the OA and control samples studied are summarised in Table S1 (supplementary material).
Preparation of cartilage explant conditioned media.

Cartilage explants were dissected into serum-free DMEM (1 ml/g cartilage) supplemented with 25 mM HEPES, penicillin (100 U/ml), streptomycin (100 μg/ml) and amphotericin (2 μg/ml). The explants were washed once and left overnight in serum-free DMEM. The following day the tissue was incubated in methionine/cysteine-free DMEM (30 min) and then in the same medium containing $[^{35}\text{S}]$methionine/cysteine (200 μCi/ml/g cartilage) for 5 h.

Precipitation of proteoglycans with cetyl pyridinium chloride (CPC).

Glycosaminoglycan content was estimated as chondroitin sulphate using the dimethyl methylene blue (DMB) assay (9). Whale and shark chondroitin sulphate (Sigma, Poole, Dorset, UK) was used as a standard. A 5 % aqueous (w/v) cetylpyridinium chloride solution was prepared and 3 mg CPC/mg GAG was added to each sample (unless otherwise stated). After 30 min at room temperature, the proteoglycan/CPC-containing precipitate was centrifuged and the supernatant removed. Samples for 2D-electrophoresis were dialysed (10 kDa cut off) against water at 4°C overnight, and lyophilized. The pellets were washed with 0.4 M sodium acetate in 90% ethanol and 90% ethanol to remove CPC and sodium acetate respectively. The residual proteoglycan-rich pellet was mixed with 4 volumes of sample buffer, boiled for 5 min and then run on a 12.5% SDS-PAGE gel.

2D-gel electrophoresis.

The lyophilized residues were dissolved in 9.5 M urea, 1 % (w/v) dithiothreitol, 2 % CHAPS and 0.5 % carrier ampholyte buffer (Amersham Biosciences) supplemented with proteinase inhibitors and loaded into 13 cm long linear pH 3-10 immobilised pH gradient (IPG) dry strip (Amersham Biosciences) by in-gel rehydration. Samples (50-100 μg) were isoelectrically focused using a Multiphor II flatbed electrophoresis system, (Amersham Biosciences) at 300 V for 1 min, then ramped to 3500 V for 1.5 h and then kept at 3500 V for 3.5 h. Prior to the
second dimension separation disulphide bonds were reduced by incubating the IPG strips for
15 min with 65 mM dithiothreitol (DTT) in equilibration buffer (2 % SDS, 6 M Urea, 30 %
v/v glycerol and 150 mM Tris pH 8.8). Free SH-groups were alkylated by treatment with 260
mM iodoacetamide in equilibration buffer for 15 min. Following equilibration, the strips were
transferred to a 12.5 % polyacrylamide gel (Laemmli (10) without stacking gel) and run at 8
mA. Gels were fixed and silver stained using a mass spectrometry compatible protocol (11).
After staining, gels were soaked for 2 h in 3% glycerol and dried, prior to visualization of
metabolically radiolabelled proteins by autoradiography.

Quantification of protein expression.
The autoradiograms were scanned using a BioRad 710 imaging densitometer, and the
autoradiographic patterns were analysed using Phoretix 2D software (version 6.01; Nonlinear
Dynamic Ltd, UK). The area and pixel intensity of each spot was measured, enabling
calculation of individual spot volumes, which were expressed as a percentage of the
integrated spot volume for the entire gel.

Mass spectrometry.
The dried gels were rehydrated and silver stained features matching radiolabelled spots were
excised as described (12). In gel trypsinolysis was performed using an Investigator Progest
(Genomic Solutions, Huntingdon, UK) robotic digestion system, as previously described
(13). Tandem electrospray mass spectra were recorded using a Q-Tof hybrid quadrupole /
orthogonal acceleration time of flight spectrometer (Waters, Manchester, UK) interfaced to a
Waters CapLC capillary chromatograph. Samples were dissolved in 0.1% aqueous formic
acid, injected onto a Pepmap C18 column (300 µm x 0.5 cm; LC Packings, Amsterdam, NL),
and eluted into the electrospray with an acetonitrile / 0.1% formic acid gradient (5% to 70 %
acetonitrile over 20 minutes).
Data dependant MS/MS acquisitions were performed on precursors with charge states of 2, 3 or 4 over a survey mass range 540-1200. Known trypsin autolysis products and keratin derived precursor ions were automatically excluded. Proteins were identified by correlation of uninterpreted tandem mass spectra to entries in SwissProt/TREMBL, using ProteinLynx Global Server (14). One missed cleavage per peptide was allowed, and the fragment ion tolerance was set to 100 ppm. Carbamidomethylation of cysteine was assumed, but other potential modifications were not considered in the first pass search. All matching spectra were reviewed manually, and in cases were the score reported by ProteinLynx global server was less than 100, additional searches were performed against the NCBI nr database using MASCOT, which utilizes a robust probabilistic scoring algorithm (15).

Isolation of chondrocytes.

Chondrocytes were isolated from the cartilage by digestion with pronase E (1 mg/ml/g cartilage) for 30 min at 37°C followed by collagenase (1 mg/ml/g cartilage) for 5 h at 37°C. The digest was strained then centrifuged at 500 x g for 8 min. Pellets were washed twice, and resuspended in DMEM containing 10% FCS supplemented with 25 mM HEPES, penicillin (100 U/ml), streptomycin (100 μg/ml) and amphotericin (2 μg/ml). Cells were counted and plated on 12-well plates, (diameter of 22.6 mm) at a density of 2.5 million cells per well (100% confluent).

Encapsulation of chondrocytes in alginate.

Chondrocytes from femoral condyle of patient with OA were isolated by incubating cartilage with pronase E (1 mg/ml) for 30 min, followed by collagenase (1 mg/ml) for overnight. The digest was strained and centrifuged at 500 g for 5 min. The pellet was washed twice with DMEM supplemented with 10% (v/v) FCS. Cells were counted and resuspended in 1.2%
(w/v) (Keltone® LV, ISP Alginates, UK) in 0.15 M NaCl at a density of 4 x 10^6 cells/ml, which was passed dropwise through a 25 gauge needle into 102 mM CaCl_2. After 10 min of polymerization, beads were washed twice in 0.15 M NaCl and finally in DMEM supplemented with 10% (v/v) FCS and HEPES. The cells were cultured for 5 weeks in the same medium in a humid atmosphere of 5% CO_2 in air at 37 °C. The medium was replaced twice weekly.

Stimulation of chondrocytes in alginate.
After 5 weeks of alginate culture, thirty beads (approximately 0.75 x 10^6 cells in 30 beads) were reseeded into a 24 well plate and cultured in 500 µl of DMEM supplemented with HEPES overnight. The following day, the beads were washed twice with the same medium and stimulated with either activin A (R&D Systems, Abingdon, UK) (100ng/ml) or TGFβ (PeproTech EC Ltd, London, UK) (10ng/ml) for 48 hours.

Western blotting for TIMP-1.
The harvested conditioned medium was precipitated with TCA and subjected to SDS-PAGE with 12% (w/v) acrylamide gel. The proteins separated in the gel were electrotransferred onto PVDF membrane and reacted with sheep anti-(human TIMP-1) (from Professor Hideaki Nagase, Kennedy Institute of Rheumatology) followed by horseradish peroxidase-conjugated rabbit anti-(sheep IgG) IgG (DAKO A/S, Denmark). Immunoreactive TIMP-1 was visualized with enhanced chemiluminescence (ECL, Amersham Biosciences, UK).

Reverse transcriptase PCR.
Chondrocytes were serum-depleted for 5 h and then stimulated for 24 h. RNA was isolated from the cells using Rneasy Mini-columns (QIAGEN Ltd, UK) and reverse-transcribed into DNA using Superscript II (Gibco). PCR amplification was performed using PuRe Taq
Ready-to-go PCR beads (Amersham Biosciences). The primers used for inhibin βA subunit were 5'-CCTCCCAAAGGATGTACCCAAC-3' (sense strand) and 5'-GTGATGATCTCCGAGGTCTGCT-3' (antisense strand). The primers were derived from the human sequence of activin βA chain (accession number NM_002192).
Results

2D-electrophoresis of proteins secreted by articular cartilage: the need for proteoglycan removal

In initial 2D-electrophoresis experiments it was found that proteins secreted by cartilage explants did not focus in the first dimension. This was probably due to the presence of highly anionic proteoglycans, particularly aggrecan, interfering with isoelectric focusing. Aggrecan was therefore removed by precipitation with the cationic detergent cetylpyridinium chloride (CPC) (16).

The GAG content of medium conditioned by culturing cartilage (as estimated with the DMB assay) varied, but was normally around 200 μg/ml for porcine and 100 μg/ml for human material. After addition of CPC, precipitates were centrifuged and both the supernatants and proteoglycan–rich pellets were analysed by 1D-electrophoresis (Fig 1a). Addition of 1 mg CPC/mg GAG removed about 80% of the GAG from porcine cartilage conditioned medium (Fig 1b) but some smaller proteins (molecular mass less than 30 kDa) were lost from the supernatant and were found in the precipitate (Fig 1a). When 2 to 4 mg CPC/mg GAG were added more than 95% of the GAG was precipitated and little silver-stainable protein was present in the pellet, although cartilage link protein was identified by HPLC MS/MS (Fig 1a arrowed). Precipitation was carried out at room temperature since non-specific co-precipitation of several proteins was observed at 4 ºC (data not shown). For routine use it was decided to add 3 mg CPC/mg GAG and the medium was left for 30 min at room temperature. After centrifugation the medium was dialysed and the proteins secreted by porcine cartilage were then well resolved by 2D-electrophoresis (Supplementary material, Fig S1 and Table S2).

2D-electrophoresis of proteins secreted from human cartilage
Human cartilage was dissected from fresh surgical samples, washed overnight with serum-free DMEM to remove extraneous proteins and then metabolically radiolabelled with $[^{35}\text{S}]$methionine/cysteine for 5 hours. CPC precipitation was carried out as described above. Altogether 12 osteoarthritic samples and 17 controls with macroscopically normal cartilage were analysed (Table 1).

Several hundred protein spots were usually observable on the silver stained gels, of which 170 were excised, digested in gel and identified by HPLC MS/MS (Supplementary material, Fig S2 and Table S3). Many of these were not chondrocyte-derived but were plasma or other proteins originating from synovial fluid and blood cells (e.g. haemoglobin and carbonic anhydrase). The autoradiographic patterns representing newly synthesised chondrocyte proteins were simpler, with between 50 and 100 well-focused protein spots (depending on the sample loading) being observable. A typical autoradiographic pattern from cultured osteoarthritic cartilage is shown in Fig 2. Nineteen radiolabelled proteins were identified by HPLC-MS/MS (Fig 2). These are listed in Table 1, together with some non-labelled cartilage proteins and several radiolabelled proteins, which were absent from the 2D pattern but were detected by 1D-electrophoresis. Many are present on the 2D-gel (Fig 2) as multiple gel spots because of glycosylation or other modifications. The reproducibility of the 2D patterns is shown by comparison of 7 autoradiograms from normal and OA joints representing a wide age range (Fig 3).

Lumican and clusterin were abundantly present in multiple isoelectric forms and hindered detection of other proteins migrating to the same region of the gel. For example, MMP-2 and MMP-3 were sometimes obscured by lumican. MMP-1 was not observed on the 2D-gels but may be hidden in the lumican cluster, since it was identified by MS after 1D electrophoresis (Table 1). Relative expression levels of these MMPs in OA and control samples were therefore difficult to assess from the 2D-gel patterns.
Expression of YKL-40 (also known as cartilage glycoprotein 39 and chitinase-3 like protein 1) is reportedly increased in OA cartilage (21, 24-25). However, we found its production variable: it was detected in 12/14 adult controls and in 4/12 OA samples. In contrast, the related protein YKL-39 was a consistent feature of the 2D patterns in both normal and OA cartilage.

Two interesting potentially regulatory molecules, inhibin βA and connective tissue growth factor (CTGF), were identified. These proteins have not previously been described in articular cartilage (see below).

**Synthesis of collagen type II is increased in cartilage explants from young or OA subjects**

In the osteoarthritic sample shown in Fig 2, two high molecular mass metabolically labelled spots (apparent Mr > 116kDa) were identified as type II collagen. The larger species was presumed to be the pro-α-chain, the smaller the processed α-chain. The type II collagen C-terminal propeptide was detected in 1-3 isoelectric forms of about 35kD (Figs 2 and 3). Procollagen C-proteinase enhancer protein was also detected, but the C-proteinase itself was not found. The OA explants (Fig 2 and 3a) were clearly synthesising and processing type II collagen.

Inspection of the 2D patterns from 12 osteoarthritic and 17 normal samples listed in Table 1 showed that the collagen II α-chain and the C-propeptide were made by most of the OA samples but not by the normal adult controls (Figs 2 and 3a,e,f). Because the pro-α-chain and α-chain spots often focused poorly, the normalised spot volume of the C-propeptide was used as a measure of type II collagen synthesis (Fig 4). The C-propeptide was a prominent feature in 9 out of 12 OA samples (0.4-6.3% of total spot volume). It was undetectable in one and only weakly present in two others (about 0.1% of total spot volume). In samples from control adult cartilage, the C-propeptide was either undetectable or comprised less than 0.3% of total
spot volume. In contrast, cartilage from two very young subjects (7 weeks and 6 years) synthesised significant amounts of collagen type II α chain and C-propeptide (Fig 3b,c). The latter was also just detectable in a further sample from a 13 year old (Fig 3d).

Newly synthesised type II collagen and its C-terminal propeptide were also prominent in the medium of cultured explants of porcine cartilage (Supplementary material Fig S1 and Table S2). The porcine cartilage was from animals that were 3-6 months old at slaughter (i.e. skeletally immature). Taken together, these results suggest that in healthy cartilage type II collagen synthesis declines with skeletal maturity but may be reactivated in OA.

**Secretion of the βA chain of activin/inhibin by OA cartilage explants**

A diagonal line of spots with molecular masses around 45 kDa was identified as pro-inhibin βA by HPLC MS/MS (Fig 2 and 3). A single spot at 14 kDa corresponded to mature inhibin βA. The increasing mass and acidity of the 45kDa spot chain is consistent with complex or hybrid type N-glycosylation, and inspection of the sequence reveal a single Asn-X-Thr N-linked glycosylation sequence (Asn 165). By contrast the fully processed form, which lacks this consensus glycosylation site, is observed as a single spot in a position consistent with its calculated mass and pI (12,976 / 7.1).

Inhibin βA chains homodimerize by disulphide bonding to form activin A, or heterodimerise with inhibin α-chains to form inhibin A. Since no inhibin α-chains or other types of β-chain were detected, presumably only activin A (a βA-βA homodimer) is present.

Proinhibin βA was secreted by all the OA explants and the mature protein was present in 10/12 samples. Production of proinhibin βA and inhibin βA in adult control samples was variable and only detected in half of the samples. Production was detectable in the samples from the three youngest subjects (7 weeks, 6 years and 13 years), (Fig 3b,c,d)
Activin A is induced by growth factors and cytokines

Activin is produced by cultured fibroblasts and keratinocytes in response to stimulation by growth factors or the inflammatory cytokines IL-1 and TNFα (17). Serum-starved monolayers of human chondrocytes were therefore treated for 24 hours with IL-1, activin A, TGFβ1, FGF-2, PDGF or EGF to identify potential mediators of pro-inhibin βA induction in cartilage. All of these agents, including activin A itself induced activin A mRNA (Fig 5a). The concentration of activin A protein in the culture medium was also increased by these stimuli: IL-1 and TGFβ caused the highest increase, consistent with their effect on the mRNA (Fig 5b). The sample containing activin A was not assayed for protein.

Activin A induces TIMP-1 protein in chondrocytes

Since activin A is a member of the TGFβ family, and TGFβ is known to induce expression of TIMP-1 in chondrocytes (18), we stimulated alginate encapsulated human articular chondrocytes with activin A or TGFβ for 48 hours. Immunoblotting showed that both stimuli increased levels of TIMP-1 protein in the culture medium (Fig 6a), and in the case of activin A, induction was concentration dependent (Fig 6b).

Discussion

This study is, to our knowledge, the first application of 2D-electrophoresis and MS to analyse proteins made by articular cartilage. Removal of sulphated proteoglycans by treatment with CPC was essential for successful 2D-electrophoresis. Between 50 and 100 radiolabelled protein spots were visible, though many of these were below the silver stain detection limit. Inevitably, since our analytical strategy entailed a deliberate removal of proteoglycans, we did not detect aggrecan, or link protein (which was, however found in the proteoglycan-rich pellet). It is likely that small proteoglycans such as biglycan and decorin were also...
precipitated with the pellet since they were not observed on the 2D-gels. Given that aggrecan and link protein were precipitated by CPC and that fibronectin and MMP-1 were not resolved on 2D-electrophoresis, we accounted for a total of 27 cartilage proteins secreted by cartilage. Two-dimensional electrophoresis has inherent limitations, particularly for analysis of hydrophobic, high molecular mass, or extremely acidic and basic proteins. Generally, 2-3 g of cartilage are needed to obtain 2D-gels from which radiolabelled proteins can be identified by mass spectrometry. In the case of OA samples it was usually necessary to use all available material so diseased tissue may sometimes be mixed with a proportion of undamaged cartilage. Hence differences between the protein expression patterns in osteoarthritic and normal cartilage may be masked by the heterogeneity of the diseased material. Gene expression in normal and osteoarthritic cartilage has been studied using microarrays and by sequencing cDNA libraries. These approaches depend on obtaining 10-20 µg of mRNA per sample, and thus require comparable amounts of cartilage to the present study. Gene expression profiling cannot predict a priori quantities of protein made, post-translational modifications or protein-protein interactions.

We have previously shown that dissection of cartilage results in activation of the extracellularly regulated kinase (ERK) pathway, due to release of basic FGF which is sequestered in the extracellular matrix (19). Dissection and explantation also transiently activates the c-jun N-terminal kinase (20). Although the explants were rested and washed overnight, the patterns of proteins synthesised by the cartilage may differ from in vivo. Cell activation on explantation could also obscure differences between osteoarthritic and normal tissue, and could induce expression of MMPs, inhibin βA/activin A and TIMP-1. Caution is thus needed when comparing the behaviour of OA and normal samples. Basic FGF was not
found on the 2D-gels of the cartilage culture medium, but it is extremely basic which would not favour its isoelectric focusing, and it is present in low concentration.

Our observation that type II collagen synthesis and processing was increased in osteoarthritic cartilage is consistent with microarray data (7), in situ hybridisation studies (21) and with early work using [3H]proline incorporation (22). In contrast, Kumar et al (23), who sequenced cDNA libraries derived from OA and normal cartilage did not observe differences in type II collagen mRNA expression. However since mRNA from several subjects was pooled, individual variation could have biased the results.

Although we consistently observed collagen type II C-terminal propeptide, procollagen C-proteinase (BMP-1) was absent from the gels. Procollagenase enhancer protein, which binds the type II collagen C-terminal propeptide and potentiates the action of procollagen C-proteinase (24), was observed at the protein level, but has not been detected in published transcriptomic studies (7,23). Collagen type VI α-chain was readily detectable, but apart from a 30 kDa unlabelled fragment of type XI, other collagens were not found.

In contrast to control cartilage from adult donors, normal cartilage from young subjects (and skeletally immature pigs) secreted collagen type II C-propeptide and α chain at similar levels to OA samples. Thus type II collagen synthesis may decline with skeletal maturity but becomes reactivated in OA.

Inhibin βA chain (a member of the TGFβ superfamily (25)) was secreted by most cultured OA samples but its production by normal cartilage was variable. Its expression may be induced by the cell activation caused by explantation and the release of bFGF (19,20). We found it could be induced in human chondrocytes by IL-1 or several growth factors including bFGF. It increased chondrocyte expression of TIMP-1. In view of its relationship to TGFβ, it is likely that activin A is anabolic for cartilage.
Inhibin and activin were originally discovered in ovarian follicular fluid. Activin stimulated but inhibin inhibited release of follicle-stimulating hormone from the pituitary cells. Activin was subsequently shown to be a powerful mesodermal inducer of embryonic ectoderm in Xenopus (26). Activin binds to heterodimeric receptor complexes (ActRI and ActRII) similar to TGFβ receptors, whose activation leads to phosphorylation of the transcription factors Smad 2 and Smad 3, which transduce the signal from the cytoplasm to the nucleus via interaction with Smad 4 (27,28). It is interesting that Smad 3 null mice displayed OA-like degenerative changes in their cartilage (29). Activin also binds to the naturally occurring glycoprotein inhibitor follistatin, (30), which we did not find.

Activin is produced at sites of inflammation (31-33) and has been shown to play a role in wound healing in mice (34). It may also be involved in bone formation since inhibin βA-chain knockout mice have craniofacial abnormalities (35). It has not previously been described in articular cartilage but induces a modest enhancement of type II collagen gene expression and proteoglycan synthesis in chondrocytes (36). In situ hybridisation and immunocytochemistry will be needed to determine if activin is expressed by osteoarthritic lesions.

A further potential regulatory molecule, CTGF was present as a low abundance 20 kDa spot in osteoarthritic samples. It was also produced by the young porcine cartilage. CTGF has previously been detected by screening cDNA libraries from OA and normal articular cartilage (23). Full length CTGF is approximately 36 kDa but cleaved forms between 10-20 kDa have been observed in serum-stimulated mouse fibroblast cultures and physiological fluids (37). It promotes proliferation and differentiation of chondrocytes in culture (38), and has been detected in mouse fracture callus (39). Since CTGF is induced by TGFβ (40) it may conceivably act downstream of activin.
Cytokine-like protein C17 mRNA was originally reported in CD34+ bone marrow stem cells (41) but the protein has not previously been detected. It is predicted to fold into four α-helices, a characteristic feature of haematopoietic cytokines and interleukins. The abundance of both cytokine like C17 and CTGF was very low, and further study is required to see if their expression differs in normal and OA cartilage.

In conclusion, we have shown that proteomic technologies can be applied to articular cartilage, and reveal potential disease-specific alterations in protein expression. Refinement of these techniques will enable definition of phenotypic changes during the progression of OA, and could also be used to evaluate chondrocytes phenotype during differentiation from stem cells for use in tissue engineering.

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Figure Legends

Fig 1. The effect of cetylpyridinium chloride (CPC) concentration upon precipitation of (a) proteins and (b) glycosaminoglycan (GAG) from cartilage explant medium. Porcine cartilage explants were cultured in DMEM (1 g/ml) for 5 h. The medium was removed and its GAG content estimated with the DMB assay. 800 μl samples were treated for 30 min at room temperature with 1-4 mg CPC/mg GAG. The precipitate was spun down and the supernatant removed. (a) Supernatants and pellets were analysed by SDS-PAGE and silver-stained. Link protein detected in the pellet is arrowed. (b) The GAG remaining in the supernatant after CPC precipitation was measured.

Fig 2. Autoradiograph of 2D-electrophoresis of OA cartilage explant medium (OA11). 2 g of cartilage was removed and washed overnight in serum-free DMEM. The following day the cartilage was pulsed with $[^{35}\text{S}]$methionine/cysteine (200μCi/ml) for 5 h. The explant medium was removed and treated with 3 mg CPC/mg GAG for 30 min at room temperature. The supernatant was removed and dialysed into water at 4 °C and lyophilized. 60 μg of proteins was used for isoelectric focusing on a pH 3-10 gradient. Focused proteins were further separated by SDS-PAGE on a 12.5% gel. Metabolically labelled protein spots were identified by mass spectrometry.

Fig 3. Autoradiographs of 2D-gels of radiolabelled proteins secreted by osteoarthritic (a) or normal (b-f) cartilage explants. Details of the cartilage samples are given in Table 1. Approximately 3 g of cartilage was used for each 2D-gel. Proteins indicated are (a) TIMP-1, (b) YKL-39, (c) collagen type II C-propeptide, (d) inhibin βA and (e) procollagen C-proteinase enhancer (f) collagen type VI.
**Fig 4.** Amounts of radiolabelled collagen type II C-terminal propeptide in normal and OA explant medium samples. The normalised spot volumes of the collagen type II C propeptide from 2D autoradiographs from fourteen normal and twelve OA samples are plotted.

**Fig 5.** Induction of activin A mRNA (a) and activin A protein (b) in human isolated chondrocytes stimulated with various factors. Primary chondrocytes in monolayer culture were serum-starved for 5 h and then stimulated with IL-1 (12.5 ng/ml), activin A (100 ng/ml), TGFβ (20 ng/ml), FGF-2 (20 ng/ml), PDGF (20 ng/ml) and EGF (20 ng/ml) for 24 h. Activin A mRNA levels were determined by RT-PCR and secreted activin A was measured in the cell medium by ELISA.

**Fig 6.** Induction of TIMP-1 protein in human chondrocytes in alginate by stimulation with activin A and TGFβ (a). Chondrocytes in alginate were either unstimulated (lane 1), stimulated with activin A (100ng/ml) (lane 2) or TGFβ (10ng/ml) (lane 3) for 48 hours. The conditioned culture medium was subjected to SDS-PAGE on a 12% gel, blotted onto a PVDF membrane and probed with an antibody against TIMP-1. In (b) chondrocytes in alginate were stimulated with increasing concentration of activin A and processed as above. Lane 1 is unstimulated, lane 2-4 is 10, 50, 100 ng/ml activin A respectively.
Table 1. Proteins identified from human osteoarthritic cartilage explant medium by 1D/2D-electrophoresis and HPLC MS/MS.

| Identified protein                                      | Electrophoresis* | Radiolabelled** |
|--------------------------------------------------------|------------------|-----------------|
| **Matrix proteins**                                     |                  |                 |
| ASPIC                                                  | 2D               | Y               |
| Cartilage intermediate layer protein (CILP)            | 2D               | N               |
| Chondroadherin                                         | 2D               | N               |
| Clusterin                                              | 2D               | Y               |
| Collagen II procα and α chain                          | 2D               | Y               |
| Collagen VI                                            | 2D               | Y               |
| Collagen XI                                            | 2D               | N               |
| COMP                                                   | 2D               | Y/N             |
| Fibromodulin                                           | 2D               | N               |
| Fibronectin                                            | 1D               | Y               |
| Lumican                                                | 2D               | Y               |
| Osteonectin                                            | 2D               | Y               |
| YKL-39                                                 | 2D               | Y               |
| YKL-40                                                 | 2D               | Y               |
| **Proteinases and inhibitors**                         |                  |                 |
| Cathepsin L                                            | 2D               | Y               |
| MMP-1                                                  | 1D               | Y               |
| MMP-2                                                  | 2D               | Y               |
| MMP-3                                                  | 2D               | Y               |
| TIMP-1                                                 | 2D               | Y               |
| TIMP-2                                                 | 2D               | Y               |
| **Regulatory molecules**                               |                  |                 |
| Complement factor B                                     | 2D               | Y               |
| CTGF                                                   | 2D               | Y               |
| Cytokine-like protein C-17                             | 2D               | Y               |
| Inhibin βA                                              | 2D               | Y               |
| Proinhibin βA                                           | 2D               | Y               |

* 1D - one dimensional; 2D - two dimensional electrophoresis  
** Y - yes; N - no
Activin

Control  IL-1  Activin  TGF-β  bFGF  PDGF  EGF

GAPDH

| Activin ng/ml | Control | IL-1 | TGF-β | bFGF | PDGF | EGF |
|---------------|---------|------|-------|------|------|-----|
| 1.2           | 16.3    | 19.8 | 6.8   | 5.2  | 3.2  |     |

Table a

Table b
**Supplementary Fig S1.** Silver stained 2D-gel of proteins secreted from IL-1 treated porcine explant medium. 6 g of porcine articular cartilage were cultured with IL-1 overnight and then pulsed with $[^{35}\text{S}]$methionine/cysteine (200 $\mu$Ci/ml) for 5 h. Explant medium was removed and treated with 3 mg CPC/mg GAG for 30 min at room temperature. The supernatant was removed and dialysed against water overnight at 4°C and lyophilized. 60 $\mu$g of protein was used for isoelectric focusing on a pH 3-10 gradient. Focused proteins were electrophoresed by SDS-PAGE on a 12.5 % gel. The gel was stained with silver, dried and exposed to film for 1 week. The gel was rehydrated and protein spots excised. Proteins were identified by HPLC MS/MS. Identified proteins corresponding to the labels on the gel are listed in Table S1.

**Supplementary Fig S2.** Silver stained 2D-gel of proteins secreted from OA cartilage explant medium (OA11). 2 g of cartilage was removed from and washed overnight in serum free DMEM. The following day the cartilage was pulsed with $[^{35}\text{S}]$methionine/cysteine (200 $\mu$Ci/ml) for 5 h. The explant medium was removed and treated with 3 mg CPC/mg GAG for 30 min at room temperature. The supernatant was removed and dialysed into water over night at 4°C and lyophilized. 60 $\mu$g of protein was used for isoelectric focusing on a pH 3-10 gradient. Focused proteins were further separated by SDS-PAGE on a 12.5 % gel. The gel was stained with silver, dried and exposed to film for 1 week. The corresponding autoradiograph is shown in Fig 2 in the paper. The gel was rehydrated and protein spots excised. Proteins were identified by HPLC MS/MS. Identified proteins corresponding to the labels on the gel are listed in Table S2.
Supplementary Figure S1

pH 3  pH 10

Collagen type II

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**Supplementary Table S1.** Human articular cartilage specimens used for proteomic analysis.

| Sample | Tissue source                                | Sex | Age |
|--------|----------------------------------------------|-----|-----|
| N1     | fem. head* (fracture)                        | f   | 82  |
| N2     | fem. head (fracture)                         | f   | 86  |
| N3     | fem. head (fracture)                         | f   | 78  |
| N4     | fem. head (fracture)                         | f   | 70  |
| N5     | fem. condyle** (post-mortem)                | ?   | 49  |
| N6     | fem. condyle (post-mortem)                  | m   | 65  |
| N7     | knee (post-mortem)                           | ?   | ?   |
| N8     | fem. head (fracture)                         | f   | 80  |
| N9     | fem. head (fracture)                         | m   | 82  |
| N10    | tibial plate (amputation for osteosarcoma)   | m   | 54  |
| N11    | fem. head (resection of epithelioma)         | m   | 40  |
| N12    | fem. condyle (amputation for osteosarcoma)   | m   | 50  |
| N13    | hum. head† (excision following avascular necrosis) | m | 32  |
| N14    | hum. head (metastatic breast cancer)         | f   | 60  |
| N15    | fem. condyle (post-mortem)                  | ?   | 7 weeks |
| N16    | fem. condyle (amputation for osteosarcoma)   | f   | 6   |
| N17    | tibial plate (amputation for osteosarcoma)   | f   | 13  |
| OA1    | fem. condyle                                | f   | 54  |
| OA2    | fem. head                                   | f   | 70  |
| OA3    | fem. head                                   | f   | 63  |
| OA4    | fem. condyle                                | m   | 65  |
| OA5    | fem. head                                   | f   | 62  |
| OA6    | fem. condyle                                | f   | 53  |
| OA7    | fem. condyle                                | f   | 83  |
| OA8    | fem. condyle                                | m   | 60  |
| OA9    | fem. condyle                                | m   | 68  |
| OA10   | fem. head                                   | f   | 83  |
| OA11   | hum. head                                   | f   | 76  |
| OA12   | hum. head                                   | f   | 72  |
| OA11   | hum. head                                   | f   | 76  |
| OA12   | hum. head                                   | f   | 72  |

N: normal; OA: osteoarthritic

* femoral head, ** femoral condyle, † humeral head
**Supplementary Table S2.** Proteins identified from a 2D-gel of porcine explant medium by HPLC-MS/MS. The gel is shown in Fig S1.

| Spot | Protein                              | Exp pI/Mw | Theor. pI/Mw | Sequence |
|------|--------------------------------------|-----------|--------------|----------|
| A1   | Connective tissue growth factor (CTGF) | 6.8/21000 | 8.38/35404   | QUGEL CTER LPSD CPPR GLCFC FOSPA NKR CPAGY SVLVD GCOCC R QUGEL CTERD PCDPH K |
| A2   | Peptidyl-prolyl cis-trans isomerase B | 10/20000  | 9.13/20158   | IGDEM IGR DVTIA DCGK TYDNF VALAT GEK DTNS QHTH TVK SITGE RPIDE NFK VIKDE MRGG DFTJ |
| A3   | Cartilage oligomeric matrix protein (COMP) | 10/20000 | 4.35/80934   | SITGF GEGLR NALWH TDGTA SQVR EQSAL QTQCL K TFRHE SEDCT SR |
| A4   | Connective tissue growth factor (CTGF) | 5.9/21000 | 8.38/35404   | SITGF GEGLR NALWH TDGTA SQVR EQSAL QTQCL K TFRHE SEDCT SR |
| A5   | Metalloproteinase inhibitor 1 (TIMP-1) | 8.8/27000 | 8.29/20690   | GFWAL GDAPD IR EPYMC TWQLR RPR FHYP AMENSY CFTYH R LQSDT HCLWT DOLLT GSDK |
| A6   | Metalloproteinase inhibitor 1 (TIMP-1) | 8.5/27000 | 8.29/20690   | GFWAL GDAPD IR EPYMC TWQLR RPR FHYP AMENSY CFTYH R LQSDT HCLWT DOLLT GSDK |
| A7   | Triosephosphate isomerase             | 7.3/28000 | 6.51/26538   | IAVAA QCYK IYOG SYTIGT CCK HYTG SDELI GQK TAPQ QAOEV HIEK VLYFL EPWYA RITKG VAHAL AEGUL VIGAI GIEK |
| A8   | Collagen alpha 1 type II C-propeptide | 7.3/28000 | 6.66/27405   | LILYA ASLQ SGOVS R |
| A9   | Haemoglobin beta chain                | 7.3/28000 | 7.25/16034   | LILYA ASLQ SGOVS R |
| A10  | Triosephosphate isomerase             | 6.6/32000 | 6.66/27405   | FVPWV NWK IAVAA QCYK IYOG SYTIGT CCK HYTG SDELI GQK TAPQ QAOEV HIEK VLYFL EPWYA RITKG VAHAL AEGUL VIGAI GIEK |
| A11  | Collagen alpha 1 type II C-propeptide | 7.2/36000 | 6.66/27405   | NLSAVL DUEAA GNLKK |
| A12  | Collagen alpha 1 type II C-propeptide | 6.5/36000 | 6.66/27405   | NLSAVL DUEAA GNLKK |
| A13  | Collagen alpha 1 type II C-propeptide | 6.3/36000 | 6.66/27405   | NLSAVL DUEAA GNLKK |
| A14  | Collagen alpha 1 type II C-propeptide | 6.2/36000 | 6.66/27405   | NLSAVL DUEAA GNLKK |
| A15  | Collagen alpha 1 type II C-propeptide | 6.1/36000 | 6.66/27405   | NLSAVL DUEAA GNLKK |
| A16  | Collagen alpha 1 type II C-propeptide | 6.1/36000 | 6.66/27405   | NLSAVL DUEAA GNLKK |
| A17  | Collagen alpha 1 type II C-propeptide | 6.1/36000 | 6.66/27405   | NLSAVL DUEAA GNLKK |
| B1   | Malate dehydrogenase                  | 6.2/36000 | 6.15/36323   | VLTG AAOQG AYSSL TSYON GSVFG K TLYGL GAAYV QK |
| B2   | L-lactate dehydrogenase B chain       | 5.8/36000 | 6.66/27405   | FTTYV LKDCG TK NLSAVL DUEAA GNLKK ALRIQ GSNVD EIRAE GSNR |
| B3   | Collagen alpha 1 type II C-propeptide | 5.4/37000 | 5.58/36481   | GLTSS INQK LKDESDE VAQLE NTAIDT LWQGK K VKEG CNLDL AR MVVES AVEYI K SLTEDE LALVL VLEDK ALRIQ GSNVD EIRAE GSNR |
| B4   | Collagen alpha 1 type II C-propeptide | 5.4/37000 | 6.66/27405   | FTTYV LKDCG TK NLSAVL DUEAA GNLKK ALRIQ GSNVD EIRAE GSNR |
| B5   | Collagen alpha 1 type II C-propeptide | 5.3/37000 | 6.66/27405   | FTTYV LKDCG TK NLSAVL DUEAA GNLKK ALRIQ GSNVD EIRAE GSNR |
| Protein Name                       | MW  | pI   |
|-----------------------------------|-----|------|
| Thrombospondin 1                  | 5.3/37000 | 4.74/129553 |
| Collagen alpha 1 (XI) chain       | 5.3/37000 | 5.08/181120 |
| B4 Collagen alpha 1 (XI) chain    | 4/38000  | 5.08/181120 |
| B5 Collagen alpha 1 (XI) chain    | 4.2/38000 | 5.08/181120 |
| B6 Annexin A8                     | 5.6/6000 | 5.56/36879 |
| Collagen alpha 1 (XI) chain       | 5.3/6000 | 4.74/129553 |
| B7 Collagen alpha 1 (XI) chain    | 7.5/26000 | 5.08/181120 |
| B8 Angiopoietin-related protein 2 | 8/26000  | 7.07/54802 |
| Glutathione S-transferase P       | 8/26000  | 8.07/23496 |
| Collagen alpha 1 (XI) chain       | 8/26000  | 5.08/181120 |
| B9 Osteronectin/SPARC             | 4.2/40000 | 4.66/32697 |
| Clusterin                         | 4.2/40000 | 5.7/48695 |
| Heat shock 70 kDa protein         | 4.2/40000 | 5.32/69199 |
| B10 Osteronectin/SPARC            | 4.4/39000 | 4.66/32697 |
| B11 Osteronectin/SPARC            | 4.6/38000 | 4.66/32697 |
| B12 Osteronectin/SPARC            | 4.1/41000 | 4.66/32697 |
| C1 Osteronectin/SPARC             | 4.1/42000 | 4.66/32697 |
| C2 Aspartate aminotransferase     | 9.7/43000 | 8.98/44664 |
| C3 Matrix metalloproteinase-1 (MMP-1) | 5.6/59000 | 5.41/51164 |
| C4 Polyubiquitin                  | 5.4/14000 | 7.13/64897 |
| C5 Polyubiquitin                  | 5.6/14000 | 7.13/64897 |
| C6 Calpactin 1 light chain        | 6.3/14000 | 6.35/10943 |
| C7 Elongation factor 1-delta      | 6.7/14000 | 4.9/31121 |
| C8 Haemoglobin beta chain         | 7.4/14000 | 7.25/16034 |
| C9 Haemoglobin beta chain         | 8/14000  | 7.25/16034 |
| Protein Name                        | Location | Value | 1st Value | 2nd Value |
|------------------------------------|----------|-------|-----------|-----------|
| Haemoglobin epsilon chain          |          | 8/14000 | 8.73/15963 |           |
| C10 Haemoglobin beta chain         |          | 7.7/14000 | 7.25/16034 |           |
| C11 Superoxide dismutase [Cu-Zn]   |          | 6.5/18000 | 6.04/15760 |           |
| Haemoglobin beta chain             |          | 6.5/18000 | 7.25/16034 |           |
| C12 Haemoglobin beta chain         |          | 6.8/19000 | 7.25/16034 |           |
| D1 Fibrogen A-alpha-chain          |          | 8.3/18000 | 6.55/47354 |           |
| Nucleoside diphosphate kinase B    |          | 8.3/18000 | 8.52/17298 |           |
| D2 Cofilin, non-muscle isoform      |          | 8.5/19000 | 8.16/18518 |           |
| D3 Phosphatidylethanolamine-binding protein | | 8.2/21000 | 7.39/20854 |           |
| D4 Peroxiredoxin 1                 |          | 7.5/21000 | 8.26/22176 |           |
| Peroxiredoxin 4                    |          | 7.5/21000 | 8.16/18518 |           |
| D5 Peroxiredoxin 5                 |          | 8.1/22000 | 8.86/22176 |           |
| Peroxiredoxin 4                    |          | 8.1/22000 | 8.86/22176 |           |
| D6 Peroxiredoxin 1                 |          | 8.9/24000 | 8.26/22176 |           |
| Peroxiredoxin 4                    |          | 8.9/24000 | 8.26/22176 |           |
| D7 Peroxiredoxin 1                 |          | 9.1/24000 | 8.86/22176 |           |
| Ig lambda chain C region           |          | 9.1/24000 | 8.86/22176 |           |
| Peroxiredoxin 4                    |          | 9.1/24000 | 8.86/22176 |           |
| D8 DJ-1 protein                    |          | 5.8/23000 | 6.33/19847 |           |
| D9 RNA-binding protein regulatory subunit |         | 6.4/23000 | 6.33/19891 |           |
| D10 Ig lambda chain C region       |          | 5.9/24000 | 6.76/11003 |           |
| Angiopoietin 1 receptor            |          | 5.9/24000 | 6.76/11003 |           |
| D11 Ig lambda chain C region       |          | 5.9/24000 | 5.73/21529 |           |
| Thioredoxin-dependent peroxide reductase |         | 6.9/24000 | 6.76/11003 |           |
| D12 Ig lambda chain C region       |          | 7.8/24000 | 6.76/11003 |           |
| E1 Superoxide dismutase [Mn], mitochondrial |     | 8.2/24000 | 6.86/22204 |           |
| E2 Glutathione S-transferase P     |          | 8.5/25000 | 8.07/23496 |           |
| E3 Thrombospondin 1                |          | 6.3/28000 | 4.74/129553 |         |
| E4 Thrombospondin 1                |          | 5.5/28000 | 4.74/129553 |         |
| Protein Name | Accession | M.Wt | P.I. | Description |
|-------------|-----------|------|-----|-------------|
| Thrombospondin 1 | E5 | 5.2/35000 | 4.74/129553 | FVFGT TPEDI LR YVANL FQKY |
| Thrombospondin 1 | E6 | 5.5/29000 | 4.74/129553 | FVFGT TPEDI LR LKPSV LEATV DWRV |
| Thrombospondin-1 | E7 | 5.3/39000 | 4.74/129553 | FVFGT TPEDI LR LKPSV LEATV DWRV |
| Inorganic pyrophosphatase | E8 | 5.3/39000 | 2.73/129553 | YVANL FQKY |
| Collagen alpha 1 type II C-propeptide | F1 | 5.3/46000 | 6.66/27405 | LKPSV LEATV DWRV |
| F-actin capping protein alpha-2 subunit | F2 | 5.3/46000 | 6.66/27405 | LKPSV LEATV DWRV |
| Type IX collagen alpha 1 chain | F3 | 5.3/39000 | 6.66/27405 | LKPSV LEATV DWRV |
| L-lactate dehydrogenase A chain | F4 | 5.3/39000 | 6.66/27405 | LKPSV LEATV DWRV |
| Fructose-bisphosphate aldolase A | F5 | 5.3/39000 | 6.66/27405 | LKPSV LEATV DWRV |
| Procollagen C-proteinase enhancer protein Annexin A8 | F6 | 5.3/39000 | 6.66/27405 | LKPSV LEATV DWRV |
| Alpha-1 acid glycoprotein | F7 | 5.3/39000 | 6.66/27405 | LKPSV LEATV DWRV |
| Actin beta chain | F8 | 5.2/48000 | 6.66/27405 | LKPSV LEATV DWRV |
| Alpha enolase | F9 | 5.4/41000 | 6.66/27405 | LKPSV LEATV DWRV |
| Protein Name                          | pI      | Molecular Weight |
|--------------------------------------|---------|------------------|
| Fibrinogen beta chain                | 5.4/45000 | 7.15/52314       |
| Fibrinogen beta chain                | 5.6/45000 | 7.15/52314       |
| Fibrinogen beta chain                | 6/45000  | 7.15/52314       |
| Ornithine aminotransferase, mt        | 6.3/50000 | 6.57/48534       |
| NADP+-dependent isocitrate dehydrogenase | 7.2/47000 | 6.53/46659       |
| Fumarylacetoacetase                  | 7.2/47000 | 6.92/46103       |
| Phosphoglycerate kinase 1            | 7.2/47000 | 8.3/44596        |
| Isocitrate dehydrogenase             | 7.4/47000 | 6.53/46659       |
| Phosphoglycerate kinase 1            | 7.4/47000 | 8.3/44596        |
| Phosphoglycerate kinase 1            | 7.8/46000 | 8.3/44596        |
| Phosphoglycerate kinase 1            | 8.6/45000 | 8.3/44596        |
| 47 kDa heat shock protein            | 9.5/53000 | 7.95/44527       |
| Similar to glucose regulated protein | 5.3/56000 | 5.3/36777        |
| Alpha-1-antitrypsin                  | 5.3/56000 | 5.54/44792       |
| Similar to glucose regulated protein | 5.5/56000 | 5.32/36177       |
| Alpha-1-antitrypsin                  | 5.5/56000 | 5.54/44792       |
| Alpha enolase                        | 6.3/54000 | 6.99/47037       |
| Beta enolase                         | 6.3/54000 | 7.33/46855       |
| Phosphopyruvate hydratase            | 6.3/54000 | 4.63/16104       |
| Alpha enolase                        | 6.6/54000 | 6.99/47037       |
| Beta enolase                         | 6.6/54000 | 7.33/46855       |
| Phosphopyruvate hydratase            | 6.6/54000 | 4.63/16104       |
**G11** Alpha enolase 7/54000 6.99/47037

**Beta enolase** 7/54000 7.33/46855

**Phosphopyruvate hydratase** 7/54000 4.63/16104

**G12** Ig heavy chain V region 7.8/55000 8.99/12975

**Alpha enolase** 7.8/55000 6.99/47037

**H1** Ig heavy chain V region 8.4/55000 8.99/12975

**Protein disulphide isomerase A3** 8.4/55000 5.78/54398

**H2** Ig heavy chain V region 8.1/55000 8.99/12975

**H3** Ig heavy chain V region 8.6/55000 8.99/12975

**H4** Ig heavy chain V region 8.8/55000 8.99/12975

**H5** Procollagen C-proteinase enhancer protein P 7/58000 7.55/45549

**Beta-2-glycoprotein 1** 7/58000 8.37/36254

**H6** Ig heavy chain V region 7/58000 8.99/12975

**Procollagen C-proteinase enhancer protein P** 7/58000 7.55/45549

**H7** Serum albumin 6/56000 5.86/66152

**Procollagen C-proteinase enhancer protein P** 6/56000 7.55/45549

**H8** Alpha-2-HS-glycoprotein 4.1/64000 5.4/36868

**H9** Alpha-1-antichymotrypsin 3 4.4/62000 5.77/22840

**Alpha-1-antichymotrypsin 3** 4.5/64000 5.77/22840

**Nucleobindin 1 (CALNUC)** 4.5/64000 5.09/51087

**Alpha-1-antichymotrypsin 3** 4.6/62000 5.77/22840

**78 kDa glucose-regulated protein** 4.6/62000 5.01/70478

**Fibromodulin** 4.6/62000 5.56/41285

**Fibromodulin** 4.7/64000 5.56/41285

**RAB GDP dissociation inhibitor alpha** 4.7/64000 5/5065

**78 kDa glucose-regulated protein** 4.7/64000 5.01/70478

**Alpha-1-antichymotrypsin 3** 4.7/64000 5.77/22840

**2A1** Serum albumin 6/65000 5.86/66152
| Protein Name | Accession | Value 1 | Value 2 |
|--------------|-----------|---------|---------|
| Serum albumin | 2A2       | 4.7/160000 | 5.86/66152 |
| Collagen alpha 1 (VI) chain | 2A3       | 4.7/160000 | 5.23/106495 |
| Pyruvate kinase, M2 isozyme | 2A4       | 8.6/63000 | 5.59/4863 |
| Serum albumin | 2A5       | 7.9/68000 | 5.86/66152 |
| Cystatin B | 2A6       | 5.7/15000 | 5.87/11130 |
| Cartilage oligomeric matrix protein (COMP) | 2A7       | 4/14000 | 4.35/89934 |
| Haemoglobin beta chain | 2A8       | 6.8/14000 | 7.25/16034 |
| Polyubiquitin | 2A9       | 7.3/14000 | 7.13/68497 |
| Haemoglobin beta chain | 2A10      | 6.7/14000 | 7.25/16034 |
| Haemoglobin alpha chain | 2A11      | 8.3/14000 | 8.76/15039 |
| Haemoglobin beta chain | 2A12      | 7.2/14000 | 7.25/16034 |
| Haemoglobin alpha chain | 2B1       | 8.5/14000 | 8.76/15039 |
| Haemoglobin alpha chain | 2B2       | 9.5/14000 | 8.76/15039 |
| Haemoglobin beta chain | 2B3       | 9.8/22000 | 7.25/16034 |
| Dihydropteridine reductase | 2B4       | 7.9/32000 | 6.9/25803 |
| Collagen alpha 1 type II C-propeptide | 2B5       | 7.3/32000 | 6.66/27405 |
| Adenylate kinase isoenzyme 2, mitochondrial | 2B6       | 7.3/32000 | 8.31/26365 |
| Glyceraldehyde 3-phosphate dehydrogenase | 2B7       | 9.4/38000 | 8.52/35707 |
| Ornithine decarboxylase | 2B8       | 9.4/38000 | 5.28/51345 |
| 38 kDa heparin-binding glycoprotein (Gp38) | 2B9       | 9.8/49000 | 9.17/42443 |
| Protein Name                     | Accession Numbers | Monotonicity | Description |
|---------------------------------|-------------------|--------------|-------------|
| Transaldolase                   | 6.1/39000         | 6.36/37540   | 6.1/39000   |
| Serum albumin                   | 6.1/39000         | 5.86/66152   | 5.38/52978  |
| Actin, cytoplasmic 2            | 5.1/45000         | 5.31/41661   |             |
| Gelsolin, plasma                | 4.9/53000         | 6.36/41470   | 6.1/39000   |
| Glutathione synthetase          | 4.9/53000         | 5.67/52384   |             |
| Protein disulphide isomerase A6 | 4.9/53000         | 4.95/46170   |             |
| Protein disulphide isomerase    | 4.3/59000         | 4.73/5223    |             |
| Pigment epithelium-derived factor | 5.7/53000         | 6.31/44056   |             |
| Transferrin (prealbumin)        | 5.7/53000         | 6.34/13929   |             |
| Alpha enolase                   | 5.7/53000         | 6.99/47037   |             |
| RAB GDP dissociation inhibitor alpha | 5.9/53000     | 5/50565      |             |
| Alpha enolase                   | 5.9/53000         | 6.99/47037   |             |
| Cytosol aminopeptidase          | 6.4/58000         | 6.29/52640   |             |
| Leucine aminopeptidase          | 6.4/58000         | 7.61/51641   |             |
| Matrix metalloproteinase-3 (MMP-3) | 4.9/62000        | 5.59/52221   | 5.82/59000  |
| Alpha-1-antichymotrypsin 3      | 4.9/62000         | 5.77/22840   |             |
| Vitamin D-binding protein       | 4.9/60000         | 5.52/1243    |             |
| Antithrombin-III                | 5/62000           | 5.95/49039   |             |
| 60 kDa heat shock protein       | 5/62000           | 5.76/1054    |             |
| Lysyl oxidase homolog 2         | 5/62000           | 5.92/8406    |             |
| Lysyl oxidase homolog 2         | 5/62000           | 5.92/8406    |             |
| Serum albumin                   | 5.7/65000         | 5.86/66152   |             |
| Prolyl 4-hydroxylase alpha subunit | 5.7/65000       | 5.75/59109   |             |
| Collagen alpha 2 (XI) chain     | 7.5/10500         | 9.22/134012  |             |
| Serotransferrin                 | 6.7/95000         | 6.93/76967   |             |
| Serum albumin                   | 6.1/95000         | 5.86/66152   |             |
| Serotransferrin                 | 7/84000           | 6.93/76967   |             |
| Inhibitor of carbonic anhydrase | 7/84000           | 5.82/75697   |             |
| Lactotransferrin                | 7/84000           | 8.67/76143   |             |
| Type IX collagen alpha 1 chain  | 9.7/14000         | 8.69/20906   |             |
### Supplementary Table S3. Proteins identified from human OA cartilage explant medium (OA11, Fig S2) by 2D-electrophoresis and HPLC-MS/MS.

| Spot | Protein                                           | Exp pl/Mw | Theor. pl/Mw | Sequence                                                                 |
|------|---------------------------------------------------|-----------|--------------|--------------------------------------------------------------------------|
| A1   | Inhibin beta A                                    | 7.5/15000 | 7.07/12976   | QFFVSFK                                                                 |
| A2   | Cytokine-like protein C17                         | 4.5/18000 | 8.8/15577    | LLQYSEPKSEKEVR                                                           |
| A3   | Cytokine-like protein C17                         | 5.8/18000 | 8.8/15577    | ALSQE HTR                                                               |
|      |                                                   |           |              | DFNLL QSVSP SEPCV                                                        |
| A4   | Tetranectin                                       | 5/20000   | 5.8/19752    | LDTLQAQVALLEKQALQTVCLCK                                               |
| A5   | Tetranectin                                       | 5.8/22000 | 5.8/19752    | CFLAFTQTKT                                                               |
| A6   | Tetranectin                                       | 5.5/22000 | 5.8/19752    | CFLAFTQTKI                                                               |
| A7   | Tetranectin                                       | 5/23000   | 5.8/19752    | NWETE ITAQPD DGGKT ENCVA LSGAANGK                                      |
| A8   | Tetranectin                                       | 4.8/23000 | 5.8/19752    | TFEA SEDC1 SR                                                         |
| A9   | Metalloproteinase inhibitor 2 (TIMP-2)            | 6.5/22000 | 6.5/21755    | TYTVGEETCTVFPCSLIPCK                                                   |
| A10  | Metalloproteinase inhibitor 2 (TIMP-2)            | 6.5/22000 | 6.5/21755    | TYTVGEETCTVFPCSLIPCK                                                   |
| A11  | Metalloproteinase inhibitor 1 (TIMP-1)            | 8.8/30000 | 8.5/20708    | TYTVGEETCTVFPCSLIPCK                                                   |
| A12  | Metalloproteinase inhibitor 1 (TIMP-1)            | 8.5/30000 | 8.5/20708    | TYTVGEETCTVFPCSLIPCK                                                   |
| B1   | Metalloproteinase inhibitor 1 (TIMP-1)            | 8/30000   | 8.5/20708    | TYTVGEETCTVFPCSLIPCK                                                   |
| B2   | Metalloproteinase inhibitor 1 (TIMP-1)            | 7.5/30000 | 8.5/20708    | TYTVGEETCTVFPCSLIPCK                                                   |
| B3   | Osteoinductive factor (OIF)                       | 5/32000   | 5/26976      | ESAYLYAR ESAYLYAR                                                       |
| B4   | Clusterin                                         | 6/34000   | 5.9/50602    | ESSSEDLFQ DRFF R                                                        |
| B5   | Clusterin                                         | 5.5/34000 | 5.9/50602    | ESAYLYAR ESAYLYAR                                                       |
| B6   | Osteoinductive factor                             | 5.5/34000 | 5/26976      | ESAYLYAR ESAYLYAR                                                       |
|      |                                                   |           |              | ESAYLYAR ESAYLYAR                                                       |
| B7   | Osteoinductive factor                             | 5.5/36000 | 5/26976      | ESAYLYAR ESAYLYAR                                                       |
| Osteoinductive factor | B8 | 5/36000 5/26976 |
|-----------------------|----|----------------|
| Clusterin             | B9 | 5/36000 5.9/50062 |
| Osteoinductive factor | B10| 5/36000 5/26976 |
| Clusterin             | B11| 4.5/36000 5/26976 |
| Osteoinductive factor | B12| 3.6/44000 5/26976 |
| Osteonectin/SPARC     |     | 3.6/44000 4.7/30666 |
| Vimentin              | C2 | 3.9/43000 5.1/46814 |
| Osteonectin/SPARC     | C3 | 3.9/43000 5.1/46814 |
| Osteoinductive factor | C4 | 4/17000 5.1/46814 |
| Vimentin              | C5 | 3.5/20000 5.1/46814 |
| Vimentin              | C6 | 4/45000 5.1/46814 |
| Osteoinductive factor | C7 | 5.3/32000 5/26976 |
| Osteoinductive factor | C8 | 4.8/34000 5/26976 |
| Clusterin             | C9 | 5/24000 5.9/50062 |
| Clusterin             | C10| 4.8/40000 5.9/50062 |
| Lamin                 | C11| 5.8/42000 6.7/48452 |
| Clusterin             | C12| 4.7/34000 5.9/50026 |
| Clusterin             | D1 | 4.9/34000 5.9/50062 |
| Inhibin beta A        | D2 | 5.8/44000 7.9/45085 |

**B8**
DFADIPNLR
IEEIRLEGNPIVLGK
LSLLEELSLAENQLLKLPVLPPK
RLDFTGNLIEDIEDGTFSK
B8 Osteoinductive factor 5/36000 5/26976
ESAYLYAR DFADIPNLR LTAKDFADIPNLR LPVPLPKLTLFNAK EEIRLEGNPIVLGK
LSLLEELSLAENQLLKLPVLPPK
RLDFTGNLIEDIEDGTFSK

**B9**
Clusterin 5/36000 5.9/50062
ESAYLYAR DFADIPNLR LTAKDFADIPNLR LPVPLPKLTLFNAK EEIRLEGNPIVLGK
LSLLEELSLAENQLLKLPVLPPK
RLDFTGNLIEDIEDGTFSK

**B10**
Clusterin 5/36000 5/26976
ESAYLYAR DFADIPNLR LTAKDFADIPNLR LPVPLPKLTLFNAK EEIRLEGNPIVLGK
LSLLEELSLAENQLLKLPVLPPK
RLDFTGNLIEDIEDGTFSK

**B11**
Clusterin 4.5/36000 5/26976
ESAYLYAR DFADIPNLR LTAKDFADIPNLR LPVPLPKLTLFNAK EEIRLEGNPIVLGK
LSLLEELSLAENQLLKLPVLPPK
RLDFTGNLIEDIEDGTFSK

**B12**
Osteoinductive factor 3.6/44000 5/26976
ESAYLYAR DFADIPNLR LTAKDFADIPNLR LPVPLPKLTLFNAK EEIRLEGNPIVLGK
LSLLEELSLAENQLLKLPVLPPK
RLDFTGNLIEDIEDGTFSK

**C1**
Osteonectin/SPARC 3.6/44000 4.7/30666
ESAYLYAR DFADIPNLR LTAKDFADIPNLR LPVPLPKLTLFNAK EEIRLEGNPIVLGK
LSLLEELSLAENQLLKLPVLPPK
RLDFTGNLIEDIEDGTFSK

**C2**
Vimentin 3.9/43000 5.1/46814
ESAYLYAR DFADIPNLR LTAKDFADIPNLR LPVPLPKLTLFNAK EEIRLEGNPIVLGK
LSLLEELSLAENQLLKLPVLPPK
RLDFTGNLIEDIEDGTFSK

**C3**
Vimentin 3.9/43000 5.1/46814
ESAYLYAR DFADIPNLR LTAKDFADIPNLR LPVPLPKLTLFNAK EEIRLEGNPIVLGK
LSLLEELSLAENQLLKLPVLPPK
RLDFTGNLIEDIEDGTFSK

**C4**
Vimentin 4/17000 5.1/46814
ESAYLYAR DFADIPNLR LTAKDFADIPNLR LPVPLPKLTLFNAK EEIRLEGNPIVLGK
LSLLEELSLAENQLLKLPVLPPK
RLDFTGNLIEDIEDGTFSK

**C5**
Vimentin 3.5/20000 5.1/46814
ESAYLYAR DFADIPNLR LTAKDFADIPNLR LPVPLPKLTLFNAK EEIRLEGNPIVLGK
LSLLEELSLAENQLLKLPVLPPK
RLDFTGNLIEDIEDGTFSK

**C6**
Vimentin 4/45000 5.1/46814
ESAYLYAR DFADIPNLR LTAKDFADIPNLR LPVPLPKLTLFNAK EEIRLEGNPIVLGK
LSLLEELSLAENQLLKLPVLPPK
RLDFTGNLIEDIEDGTFSK

**C7**
Osteoinductive factor 5.3/32000 5/26976
ESAYLYAR DFADIPNLR LTAKDFADIPNLR LPVPLPKLTLFNAK EEIRLEGNPIVLGK
LSLLEELSLAENQLLKLPVLPPK
RLDFTGNLIEDIEDGTFSK

**C8**
Osteoinductive factor 4.8/34000 5/26976
ESAYLYAR DFADIPNLR LTAKDFADIPNLR LPVPLPKLTLFNAK EEIRLEGNPIVLGK
LSLLEELSLAENQLLKLPVLPPK
RLDFTGNLIEDIEDGTFSK

**C9**
Clusterin 5/24000 5.9/50062
ESAYLYAR DFADIPNLR LTAKDFADIPNLR LPVPLPKLTLFNAK EEIRLEGNPIVLGK
LSLLEELSLAENQLLKLPVLPPK
RLDFTGNLIEDIEDGTFSK

**C10**
Clusterin 4.8/40000 5.9/50062
ESAYLYAR DFADIPNLR LTAKDFADIPNLR LPVPLPKLTLFNAK EEIRLEGNPIVLGK
LSLLEELSLAENQLLKLPVLPPK
RLDFTGNLIEDIEDGTFSK

**C11**
Lamin 5.8/42000 6.7/48452
ESAYLYAR DFADIPNLR LTAKDFADIPNLR LPVPLPKLTLFNAK EEIRLEGNPIVLGK
LSLLEELSLAENQLLKLPVLPPK
RLDFTGNLIEDIEDGTFSK

**C12**
Clusterin 4.7/34000 5.9/50026
ESAYLYAR DFADIPNLR LTAKDFADIPNLR LPVPLPKLTLFNAK EEIRLEGNPIVLGK
LSLLEELSLAENQLLKLPVLPPK
RLDFTGNLIEDIEDGTFSK

**D1**
Clusterin 4.9/34000 5.9/50062
ESAYLYAR DFADIPNLR LTAKDFADIPNLR LPVPLPKLTLFNAK EEIRLEGNPIVLGK
LSLLEELSLAENQLLKLPVLPPK
RLDFTGNLIEDIEDGTFSK

**D2**
Inhibin beta A 5.8/44000 7.9/45085
ESAYLYAR DFADIPNLR LTAKDFADIPNLR LPVPLPKLTLFNAK EEIRLEGNPIVLGK
LSLLEELSLAENQLLKLPVLPPK
RLDFTGNLIEDIEDGTFSK
D3 Inhibin beta A 6/44000 7.9/45085 RPVDT QPVPK SELLE SEKVY DAR SWHYF PPSS SQQR VGENG YVEIE DIDIGR
D4 Inhibin beta A 6.4/43000 7.9/45085 RPVDT QPVPK LLDDQ KSSLD VR SWHYF PPSS SQQR VGENG YVEIE DIDIGR IACQ CJESG ASLVL LGK AEMNE LMEQT SEIT FAESG TAR
D5 Inhibin beta A 6.6/41000 7.9/45085 AEVWL FLKVP K SELLE SEKVY DAR SWHYF PPSS SQQR VGENG YVEIE DIDIGR KSTWH YPYS SQQR IACQ CJESG ASLVL LGK
D6 Inhibin beta A 6.8/41000 7.9/45085 SELL SEK RPVDT QPVPK LLDDQ KSSLD VR SWHYF PPSS SQQR VGENG YVEIE DIDIGR IACQ CJESG ASLVL LGK
D7 Inhibin beta A 7/39000 7.9/45085 SELL SEK RPVDT QPVPK LLDDQ KSSLD VR SWHYF PPSS SQQR VGENG YVEIE DIDIGR IACQ CJESG ASLVL LGK AEMNE LMEQT SEIT FAESG TAR
D8 Inhibin beta A 7.3/39000 7.9/45085 SELL SEK RPVDT QPVPK LLDDQ KSSLD VR SWHYF PPSS SQQR VGENG YVEIE DIDIGR IACQ CJESG ASLVL LGK
D9 YKL-39/ Chitinase-3 like protein 2 7.5/39000 7.2/40871 LLDDQ KSSLD VR SWHYF PPSS SQQR VGENG YVEIE DIDIGR IACQ CJESG ASLVL LGK
D10 YKL-40/gp-39/Chitinase-3 like protein 1 8.2/41000 8.65/40476 QLLLS AALSA GK THGF DLGLA WLYPG R EAGTL AYYEI CDFLR SFTLA SSETG VGAPI SGPGI PGRFT
D11 YKL-40/gp-39/ Chitinase-3 like protein 1 8.6/41000 8.65/40476 QLLLS AALSA GK THGF DLGLA WLYPG R EAGTL AYYEI CDFLR SFTLA SSETG VGAPI SGPGI PGRFT
Fructose-bisphosphate aldolase 8.6/41000 8.4/36016
D12 Alpha-1-antitrypsin 4/63000 5.3/44324 QLLLS AALSA GK THGF DLGLA WLYPG R EAGTL AYYEI CDFLR SFTLA SSETG VGAPI SGPGI PGRFT
E1 YKL-40/Gp-39/ chitinase-3 like protein 1 9/40000 8.65/40476 QLLLS AALSA GK THGF DLGLA WLYPG R EAGTL AYYEI CDFLR SFTLA SSETG VGAPI SGPGI PGRFT
E2 YKL-40/Gp-39/ chitinase-3 like protein 1 9/40000 8.65/40476 QLLLS AALSA GK THGF DLGLA WLYPG R EAGTL AYYEI CDFLR SFTLA SSETG VGAPI SGPGI PGRFT
E3 Matrix metalloproteinase-3 (MMP-3) 5.2/58000 5.7/52221 QLLLS AALSA GK THGF DLGLA WLYPG R EAGTL AYYEI CDFLR SFTLA SSETG VGAPI SGPGI PGRFT
E4 Matrix metalloproteinase-3 (MMP-3) 4.8/59000 5.7/52221 QLLLS AALSA GK THGF DLGLA WLYPG R EAGTL AYYEI CDFLR SFTLA SSETG VGAPI SGPGI PGRFT
E5 Matrix metalloproteinase-3 (MMP-3) 4.4/59000 5.7/52221 QLLLS AALSA GK THGF DLGLA WLYPG R EAGTL AYYEI CDFLR SFTLA SSETG VGAPI SGPGI PGRFT
E6 Matrix metalloproteinase-2 (MMP-2) 4.5/70000 6.4/51844 QLLLS AALSA GK THGF DLGLA WLYPG R EAGTL AYYEI CDFLR SFTLA SSETG VGAPI SGPGI PGRFT
E7 ASPIC 4/84000 4.95/68434
Lumican 4/82000 6.17/36660
E8 Lumican 4.5/53000 6.17/36660
Collagen alpha 1 type VI 4.5/130000 5.23/106495
E9 Complement factor B 5.8/90000 6.66/83000
E10 Collagen alpha 1 type VI 4.5/53000 6.17/36660
Kinesin-like protein 9.3/150000 5.42/138630
E11 Complement factor B 6.7/90000 6.66/83000
E12 Complement factor B 6.2/90000 6.66/83000
E13 Complement factor B 6.7/88000 6.66/83000
F1 Complement factor B 6.9/61000 6.66/83000
F2 Preursor polypeptide (AA-14 to 747) 9.3/150000 6.57/83881
F3 Putative DNA/chromatin binding motif 5.5/170000 5.90/144676
F4 Cartilage intermediate layer protein (CILP) 7.5/60000 8.73/132538
F5 Cartilage intermediate layer protein (CILP) 7.8/60000 8.73/132538
F6 Cartilage intermediate layer protein (CILP) 8.2/59000 8.73/132538
F7 Cartilage intermediate layer protein (CILP) 8.7/59000 8.73/132538
F9  Cartilage intermediate layer protein (CILP) 8.8/61000 8.73/132538
VPGSC CR INPLS CNYVR TFLVG NLIEB CTGSDS SGAHFR SNVGV ALTTFN CVER FNPNA KPVPLYNK BDPQ NV3AA CLEFK FLPSE EQOQVISYVNLIEPR QSAFQ YLQST FAQPS AAGTVQGR

F10  Cartilage intermediate layer protein (CILP) 9/62000 8.73/132538
VPGSC CR TAEALG IR VPGSC SCR IVGPL EVNVR TFLVNLIEB ACEEA PSRFA AAIFR FNPNA KPVPLYNK BDPQ NV3AA CLEFK FLPSE EQOQVISYVNLIEPR QSAFQ YLQST FAQPS AAGTVQGR

F11  Cartilage intermediate layer protein (CILP) 9.2/62000 8.73/132538
VPGSC CR TAEALG IR VPGSC SCR IVGPL EVNVR TFLVNLIEB ACEEA PSRFA AAIFR FNPNA KPVPLYNK LSVNR YEQSG CNLDS AR G3 L-lactate dehydrogenase 8.2/34000 8.46/35922 GALQN IIPAS TGAAK

F12  Golgin-160 8.6/50000 5.74/66349
LOSEL KELR EAADA ELGQL R HLVQA LQASL EK QELMQ VHGEK R KGTRL LGQSN AALR VIELE DGE5 SR ELEGL QOQLQ NVK EREDM ETHLQ SLOFD K ELQOK KLQAE ADDLQ FR

G1  Golgin-160 9.5/54000 5.74/66349
LOSDL TSAQK EAADA ELGQL R QELMQ VHGEK R LGSDL TSAQK EMK VLELE DGE5 SR ELEGL QOQLQ NVK EREDM ETHLQ SLOFD K ELQOK KLQAE ADDLQ FR

G2  L-lactate dehydrogenase 8.6/34000 8.46/36557
VIQSG CNLDS AR QHDAE VDATL K FTYTA LKDG FK NSKAY IDEAA GNLK K SLNOQ JEBST ALLIQ GNDVE ER

G3  L-lactate dehydrogenase 8.2/34000 8.46/36557
VIQSG CNLDS AR QHDAE VDATL K FTYTA LKDG FK

G4  Collagen alpha 1 type II C-propeptide 7/33000 6.66/27405
QDEAE YDATL K FTYTA LKDG FK

G5  Collagen alpha 1 type II C-propeptide 6.6/33000 6.66/27405
QHDAE VDATL K FTYTA LKDG FK

G6  Glyceraldehyde 3-phosphate dehydrogenase 8.2/36000 8.58/35922 GALQN IIPAS TGAAK

G7  Glyceraldehyde 3-phosphate dehydrogenase 8.4/36000 8.58/35922 GALQN IIPAS TGAAK

G8  Glyceraldehyde 3-phosphate dehydrogenase 8.7/36000 8.58/35922 GALQN IIPAS TGAAK

G9  Glyceraldehyde 3-phosphate dehydrogenase 8.8/36000 8.58/35922 GALQN IIPAS TGAAK

G10  Glyceraldehyde 3-phosphate dehydrogenase 9.2/36000 8.58/35922 GALQN IIPAS TGAAK

G11  Procollagen C-proteinase enhancer protein P 6.2/50000 7.55/45549
ATSIG EHOQC GBER E K FCGT RPAL VAPON QTBLR

G12  Procollagen C-proteinase enhancer protein P 6.5/47000 7.55/45549
ATSIG EHOQC GBER E K FCGT RPAL VAPON QTBLR

H1  Procollagen C-proteinase enhancer protein P 6.8/47000 7.55/45549
ATSIG EHOQC GBER E K FCGT RPAL VAPON QTBLR

H2  Procollagen C-proteinase enhancer protein P 7.3/47000 7.55/45549
ATSIG EHOQC GBER E K FCGT RPAL VAPON QTBLR
H3  Procollagen C-proteinase enhancer protein P 7.5/47000 7.55/45549
H4  Procollagen C-proteinase enhancer protein P 9.5/19000 7.55/45549
H5  Extracellular superoxide dismutase [Cu-Zn] 5.7/31000 6.32/24162
H6  Extracellular superoxide dismutase [Cu-Zn] 6/31000 6.32/24162
H7  Extracellular superoxide dismutase [Cu-Zn] 6.5/30000 6.32/24162
H8  Carbonic anhydrase 6.8/29000 6.63/28739
H9  Carbonic anhydrase 7/29000 6.63/28739
H10 Complement factor D 7.2/24000 6.85/24404
H11 Complement factor D 7.2/23000 6.85/24404
H12 Complement factor D 7.5/24000 6.85/24404
2A1 Biliverdin reductase B 7.7/23000 7.13/22119
2A2 Phosphatidylethanolamine-binding protein 7.8/22000 7.43/20925
2A3 Serum amyloid P 5.2/27000 6.12/23528
2A4 Cartilage oligomeric matrix protein (COMP) 4.3/31000 4.35/80394
2A5 Cartilage oligomeric matrix protein (COMP) 9.6/21000 4.35/80394
2A6 α-1-microglobulin-bikunin 4.5/32000 6.32/35528
2A7 Cartilage intermediate layer protein (CILP) Cartilage oligomeric matrix protein (COMP) 4.5/32000 8.73/132538
2A8 Peroxiredoxin 2 5.3/225000 5.66/21891
2A9 Peroxiredoxin 2 7.6/22000 5.66/21891
|   | Description                                      | MW    | Accession Numbers       |
|---|--------------------------------------------------|-------|-------------------------|
| 2A10 | Destrin (Actin-depolymerizing factor)            | 8/18000 | 8.06/18505              |
| 2A11 | Transhyretin (prealbumin)                        | 5.2/16000 | 5.35/13761              |
| 2A12 | Peptidyl-prolyl cis-trans isomerase B            | 9.7/20000 | 9.25/20289             |
| 2B1  | Peptidyl-prolyl cis-trans isomerase              | 8/17000 | 7.82/17881              |
| 2B2  | Chain A, zinc dependent dimers                   | 9.2/24000 | 9.1/19561              |
|      | Type XVIII collagen                              | 9.2/24000 | 9.3/17732              |
| 2B3  | Haemoglobin alpha chain                         | 9.3/14000 | 8.73/15136             |
| 2B4  | Haemoglobin alpha chain                         | 8.7/14000 | 8.73/15136             |
| 2B5  | Haemoglobin alpha chain                         | 8.5/14000 | 8.73/15136             |
| 2B6  | Lysozyme C                                      | 9.5/14000 | 9.38/16537             |
| 2B7  | Profilin 1                                       | 8.8/14000 | 8.47/14923             |
| 2B8  | Haemoglobin delta chain                         | 8/14000  | 7.97/15924              |
| 2B9  | Haemoglobin beta chain                          | 7.5/14000 | 6.81/15867             |
| 2B10 | Haemoglobin beta chain                          | 7.2/14000 | 6.81/15867             |
| 2B11 | Haemoglobin beta chain                          | 6.7/14000 | 6.81/15867             |
| 2B12 | Haemoglobin delta chain                         | 6.0/14000 | 7.97/15924             |
| 2C1  | Haptoglobin-1                                    | 5.5/20000 | 5.23/9192              |
| 2C2  | Retinoic acid receptor responder protein 2       | 9/18000  | 8.99/16856             |

Note: MW = Molecular Weight
2C3 Retinoic acid responder protein 2 9.3/18000 8.99/16856
     KCLAC IK
     LVHCP BETOV LR
     EAEHH QETQC LR
     AGREDP HSFYF PQQA FSK

2C4 Thrombospondin 4 N-terminal domain 9.8/21000 9.23/29230
     KPOGDF LEELK
     AFAGP SQFPE TIELR
     KPOGDF LEELK LVVR
     GAGSL ELYLD CQVDQ SVHNL PR

2C5 Ribonuclease pancreatic precursor 8.8/21000 8.98/14574
     VPNCA TR
     CKPVN TVHEI PVLDF QNVCF QEK

2C6 Glutathione S-transferase 5.2/23000 5.44/23224
     ASCLY GQLPK
     FPPTV VYFPV R
     FQDGDL LTYLQ SNTIL R
     ALPQP LKPEE TLLSQ NOQGK

2C7 Prolargin 5.2/29000 9.45/41646
     KVPV1PPR
     NQLEE VPSAL PR
     YLEKL PGVLF LYMKEK
     EYCCPY PFDFPS ALYCD SR

2C8 Prolargin 5.7/29000 9.45/41646
     KVPV1PPR
     NQLEE VPSAL PR
     YLEKL PGVLF LYMKEK
     EYCCPY PFDFPS ALYCD SR

2C9 Phosphoglycerate mutase 6.3/26000 6.67/28803
     HYGGL TGLNK
     ALEPW NEEIN POIK
     SYDVP PPMMF PIPHF VSNIS K
     YADLT EDQQL SCSEL KDI1 A!

2C10 Ig kappa chain region 8.5/24000 5.58/11608
     SGTAS YVCCL NNFFP R
     VYACE VTHQG LSSPV TK
     TVAAP SSVIF PPDSE QLJK

2C11 Ig kappa chain region 9/24000 5.58/11608
     SGTAS YVCCL NNFFP R
     TVAAP SSVIF PPDSE QLJK

2C12 Chondroadherin 9.4/29000 9.43/38271
     ASRPD ATCAS PAC
     SIPDN AFOQF GR
     FSQGA FLOVT TLK
     VVEEL KLSIN PLK
     YLELT WLONT NLEK
     GLISSP LYNLF ILQDN NNNK
     FVHDQ NQQIS YPSAI LSK
     LQNPQ SNFFP DLSELT LALTNPWK
     AAGAF DTIEL TLYL DHNKV TELPR

2D1 Chondroadherin 9.5/33000 9.43/38271
     LRVEE ELK
     ASRPD ATCAS PAK
     SIPDN AFOQF GR
     NQISS YPSAI LSK
     QLIEE YLSIN DIR
     YLELT WLONT NLEK
     GLISSP LYNLF ILQDN NNNK
     LQNPQ SNFFP DLSELT LALTNPWK
     AAGAF DTIEL TLYL DHNKV TELPR

2D2 Annexin II 8.6/33000 7.65/38472
     TNQEL GEINR
     GVDEV TVNLI LTNR
     SASLG HLETV ILGLL K
     GLGTD EDLLE EECHS R
     DLYDA GVR
     QSAF AYOR
     TNQEL GEINR
     SLYYY SQFFT K
     GVDEV TVNLI LTNR
     TKGVD ETVIV NLTIN R
     RAEDG SVDYP ELIDQ DAR
     AYTNF DAERD ALNIE TAK

2D3 Annexin II 7.7/34000 7.65/38472

2D4 Complement C4 6.8/32000 6.37/33073
     LLATL CSAEV COAC GKPGR
     SFVNI VAK
     BCSCT TNQIC K
     QHYPF EDQGSDR
     FNTFI HEDIW NIR
     YALYD ATYCL K
     KEDLY FIFWA PESAP LK

2D5 Hypothetical 187.1 kDa protein in OGG1-CNA2 4.4/14000 8.16/187132
     TDV1 NTTQK
     QDQ1 SQILQ K
     CYKLAS EGLK
     ENQAL QTVCL R
     NSDII NAQDY YGKR
     LWTVE NALKE IQALF TVCLR
     ESNQP VVkeywords: SIK
     ADDLG KGGNE ESTK
     GDGPV QGIIN FEQK
     AVCVL KGDGP VQGII NFEQK

2D6 Ribonuclease 4 9.3/17000 9.18/13823
     VSFLS ALEEY TK
     DYVSQ FEGSA LGK
     VSKDLA TTYYD VLIK
     LLDNW DSVTS TFSK

2D7 Cofilin 8.4/19000 8.22/18502
     AKPAL EDLR
     ATEHL STTSL K
     QQGPL VLEK S
     VQYDL DDFQK
     TILAP VSYEL R
     VSFLS ALLEEY TK
     DYYSQ FEGSA LGK

2D8 C-type lectin 7.5/18000 9.15/19840
     AEQOE GARI
     AKPAL EDLR
     ATEHL STTSL K
     QQGPL VLEK S
     VQYDL DDFQK
     TILAP VSYEL R
     VSFLS ALLEEY TK
     LDGIL KDSLP LQTV
     VSKDLA TTYYD VLIK
     LLDNW DSVTS TFSK

2D9 Superoxide dismutase [Cu-Zn] 5.5/19000 8.83/19995
     ESGRP KQKWG SIR
     ADDLG KGGNE ESTK
     GDGPV QGIIN FEQK
     AVCVL KGDGP VQGII NFEQK

2D10 Apolipoprotein A-1 4.8/23000 5.27/28078
     AEQOE GARI
     AKPAL EDLR
     ATEHL STTSL K
     QQGPL VLEK S
     VQYDL DDFQK
     TILAP VSYEL R
     VSFLS ALLEEY TK
     DYYSQ FEGSA LGK

2D11 Apolipoprotein A-1 4.5/23000 5.27/28078
     AEQOE GARI
     AKPAL EDLR
     ATEHL STTSL K
     QQGPL VLEK S
     VQYDL DDFQK
     TILAP VSYEL R
     VSFLS ALLEEY TK
     LDGIL KDSLP LQTV
     VSKDLA TTYYD VLIK
     LLDNW DSVTS TFSK
| Protein Name                  | Mass (kDa) | P.I. |
|------------------------------|-----------|-----|
| 2D12 Triosephosphate isomerase | 6.7/24000 | 7.09/26581 |
| 2E1 Triosephosphate isomerase | 7/24000   | 7.09/26581 |
| 2E2 Complement C1q subcomponent, B chain | 9.1/30000 | 8.85/23741 |
| 2E3 Collagen alpha 1 (XI) chain | 7.2/29000 | 9.1/97667 |
| 2E4 Annexin V                 | 4.3/33000 | 4.94/35805 |
| 2E5 Annexin V                 | 8.3/32000 | 4.94/35805 |
| 2E6 Fibrogen beta chain       | 5.3/40000 | 7.95/50762 |
| 2E7 Pigment epithelium-derived factor | 6.7/42000 | 6.56/40071 |
| 2E8 Pigment epithelium-derived factor | 5.1/50000 | 6.56/40071 |
| 2E9 Pigment epithelium-derived factor | 5.3/49000 | 6.56/40071 |
| 2E10 Pigment epithelium-derived factor | 5.6/48000 | 6.56/40071 |
| 2E11 Pigment epithelium-derived factor | 5.7/47000 | 6.56/40071 |
| 2E12 Pigment epithelium-derived factor | 5.8/46000 | 6.56/40071 |
| 2F1 Phosphoglycerate kinase   | 8.7/43000 | 8.3/44596 |
| 2F2 Serine protease HTRA1     | 7.3/55000 | 7.89/49048 |
| 2F3 Ig gamma-2 chain C region | 7.6/55000 | 7.66/35884 |
| 2F4 Complement C3 b-chain     | 7.2/68000 | 8.7/70420 |
| 2F5 Serotransferrin           | 6.9/71000 | 6.7/75181 |
| 2F6 Serotransferrin           | 6.7/72000 | 6.7/75181 |
| Protein Name | Accession Numbers | Mass (kDa) | pI | Other Information |
|--------------|-------------------|------------|----|-------------------|
| Serotransferrin | 2F7 | 6.4/73000 | 6.7/75181 | KPVEE YANCH LAR EDQTF FYAV AVVK NLMK EVYEL CCLKT R EILD WSLLQ ALQIF FK SAGWN IPGDL LCDI PEPR |
| Serotransferrin | 2F8 | 6.5/74000 | 6.7/75181 | APNHA VYTR KASYLDCIR SKDL TWNKL K SNPS DPSS ACVK MYLG EYXTA IR CSTSS LLEAC TFR EKLF LDRY LC LAK SAGWN IPGDL LCDI PEPR |
| Fibromodulin | 2F9 | 3.6/50000 | 5.57/40988 | YLPFV PSR SLIL DSNY HERK |
| Fibromodulin | 2F10 | 3.5/60000 | 5.57/40988 | YLPFV PSR SLIL DSNY HERK |
| Alpha-1B glycoprotein | 2F11 | 4.6/75000 | 5.65/51940 | |
| Hemopexin | 2F12 | 5.2/73000 | 6.43/49295 | |
| Annexin I | 2G1 | 6.5/35000 | 6.64/38583 | |
| Related to glycerol-3-phosphate dehydrogenase | 2G2 | 6.7/38000 | 7.6/76907 | |
| H factor (complement)-like | 2G3 | 6.8/39000 | 7.39/37650 | |
| Actin, aortic smooth muscle (a-actin 2) | 2G4 | 4.7/43000 | 5.24/41774 | |
| Procollagen C-endopeptidase enhancer | 2G5 | 7.6/52000 | 7.41/47972 | |
| Hypothetical 72.2 kDa protein | 2G6 | 8/51000 | 04/72247 | |
| Hypothetical 72.2 kDa protein | 2G7 | 9.4/54000 | 04/72247 | |
| Hypothetical 72.2 kDa protein | 2G8 | 8.9/53000 | 8.04/72247 | |
| Ig gamma-4 chain C region | 2G9 | 8/54000 | 7.81/35940 | |
| Beta-2-glycoprotein I | 2G10 | 6.6/57000 | 8.37/36254 | |
| Ig gamma-1 chain C region | 2G11 | 8.2/90000 | 8.46/36105 | |
| Polynucleotide nucleotidytransferase | 2G12 | 5.3/22000 | 5.3/79170 | |
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

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