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Title page
Peptidomic Analysis of Cartilage and Subchondral bone in OA patients

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Abstract

Background:

The objective of this study was to develop a method for directly analyzing osteochondral samples straight out of the operating room without cell culturing, thereby enabling identification of potential peptide biomarkers to better understand the mechanisms involved in the development of osteoarthritis and pain.

Material and Methods:

Osteochondral plugs from wounded and macroscopically non-wounded zones of the femur condyle were collected from 6 patients with manifest osteoarthritis (OA) undergoing total knee arthroplasty (TKA). The samples were demineralized and supernatant was collected and isotopically marked with Tandem Mass Tag (TMT) labeling and analyzed using liquid chromatography coupled with tandem mass spectrometry LC-MS/MS.

Results:

Using peptidomics, 6292 endogenous peptides were identified. Five hundred sixty six peptides (8 identified endogenous peptides) differed significantly (p-value 0.10) from wounded zones compared to non-wounded zones.

Conclusion:

This pilot study shows promising results for enabling peptidomic analysis of cartilage and bone straight out of the operating room. With further refinement, peptidomics can potentially become a diagnostic tool for OA, and improve the knowledge of disease progression and genesis of pain.

Keywords: Cartilage/ Mass spectrometry/ Neuropeptides/ Osteoarthritis/ Pain

Introduction

In osteoarthritis (OA), pain is the main symptom and the major cause for seeking medical care

[1-3]. Yet, the field of pain mechanisms in OA is poorly understood and largely unexplored. Studies suggest pain is affected both by mechanisms involving neuropeptide signaling [4, 5], and peripheral and central sensitization [6, 7], but also by psychological factors altering the pain perception [8]. With such complexity the profession calls for more effective guidelines in the determination of which patient category can be helped by surgical intervention.

The defined clinical and radiological criteria used today to diagnose OA have generally poor sensitivity and neither visualize the onset and early signs of disease nor predict disease progression [9-11]. Many patients are, in early stages of OA, asymptomatic and when diagnosed they already have extensive cartilage deterioration. Moreover, in OA the cartilage pathology is widely discussed but there is an indication that subchondral bone changes play an equally important role in disease progression [12, 13].

Given the low sensitivity of current diagnostic methods there has been an increasing interest in findings biomarkers to detect pathological developments in the osteoarthritic joint, predominantly by analyzing synovial fluid, serum and plasma [14-17]. To date, due to the high mineral composition of bone and the dominance of collagen in the extracellular matrix of cartilage, traditional extractions protocols cannot be applied. Solid bone and cartilage tissue have therefore been difficult to study using protein and peptide analysis methods.

Peptidomics, by which endogenous protein fragments are characterized and quantified by mass spectrometry, has already been shown to play an increasingly important role in finding biomarkers in fields such as cancer and neurodegenerative diseases [18, 19].

Peptides play a key role in many regulatory processes and there are both hormones and signalling molecules that are active as endogenous peptides, the largest group being neuropeptides.

In this pilot study we explore the possibility to perform peptidomic analysis directly on knee tissue samples to identify peptides that may be involved in pain signaling and also serve as biomarkers of OA. We developed a method for peptide extraction and analysis of endogenous peptides from osteochondral biopsies taken from wounded and macroscopically non-wounded cartilage areas in osteoarthritic knee tissue samples for subsequent analysis by liquid chromatography mass spectrometry (LC-MS) using the Tandem Mass Tag (TMT) technique for quantification. The method introduces the possibility of broadening the search and identification of potential biomarkers as well as extending our knowledge of pain and pathological mechanism involved in OA.

Materials and methods

Selection of samples

The samples used in the present study were de-identified left-over tissues from 6 patients with OA who underwent Total Knee Replacement(TKR) surgery at the Department of Orthopaedics at Sahlgrenska University Hospital, Gothenburg, Sweden. Tissue sampling was approved by patients and followed a procedure approved by the Ethics Committee at University of Gothenburg.

Peptide extraction

Tissue samples were collected during surgery and immediately taken to the laboratory for biopsy taking. An osteochondral plug (about 10 mm length, 2 mm diameter) was drilled out manually from femur condyle samples using a T-Lok Bone Marrow Biopsy needle (Argon Medical Devices, USA).

Samples were evaluated by an orthopedic surgent with large knowledge of osteochondral damages. Three samples were taken from a macroscopically healthy area of the *lateral* femur condyle with unwounded cartilage (UOA) and three samples were taken from an area with severe osteoarthritis and wounded cartilage (WOA). Samples were weigh adjusted and stored at -80°C in Eppendorf Low bind tubes (Eppendorf, Germany).

Peptide extracts were prepared by demineralizing the samples using 1.2 M HCl and 20% Acetonitrile (v/v) over night at 4°C on a rolling mixer. The supernatant was collected after centrifugation at 14,000 x g for 10 min at 4°C. Five microliters were used for Bradford Protein Assay *(BIO-RAD Bradford Quick Start Protein Assay System,* Bio-Rad Laboratories Inc.USA). The samples were volume adjusted to the same protein concentration. Sample volumes corresponding to 10 µg protein was transferred to 1.5 ml Eppendorf LoBind tubes and lyophilized. Aliquots of 1 M tri-ethyl ammonium bicarbonate (TEAB, 17 µl), 8 M Guanidium hydrochloride (Gua-HCl, 50 µl), and water (100 µl) were added and the samples and vortexed. TCEP (200 mM, 4 µl) was added and the samples were incubated at 55°C while shaking. After letting samples cool to room temperature, 400 mM iodoacetamide (4 µl) was added and the samples were incubated for 30 min at room temperature in the dark. TMT reagents source (0.8 mg) were dissolved in acetonitrile (41 µl). TMT reagent solution (15.3 µl) (Thermo Fisher Scientific, USA) was added to each sample followed by incubation for 1 h at room temperature while shaking. The labeling reaction was quenched by addition of

5% hydroxylamine (v/v, 9.5 µl) and incubation for 20 min at room temperature. The samples were combined into TMT 6-plex sets, each set consisting of three samples of tissue from unwounded areas and three samples from osteoarthritis tissue. MWCO-30 kDa ultrafiltration devices (Vivacon 2, 30 MWCO HY, VNO2H21) were washed by loading a solution of 100 mM TEAB and 3 M Gua-HCl and centrifuging at 2,500 x g for 60 min at room temperature. Samples were spun through the filter devices and the flow-through, containing the peptide fraction, was collected. An aliquot of 50 mM ammonium bicarbonate was spun through and combined with the peptide extract to improve yield. Water (1.2 ml) and 10% trifluoroacetic acid (TFA; v/v, 200 µl) were added to the peptide extracts to acidify the samples to pH<3. The peptide extracts were desalted by SPE (SEP-PAK C_{18}, Waters) operated using a vacuum chamber. The SPE cartridges were washed with 80% acetonitrile, 0.1% TFA (Buffer B) (v/v, 1 ml) and equilibrated with 0.1% TFA (Buffer A) (2 x 1 ml). After loading the samples, the cartridges were washed with Buffer A (2 x 1 ml) and subsequently eluted with Buffer B (1 ml). The elutes were lyophilized by vacuum centrifugation and stored at -80°C pending LC-MS analysis.

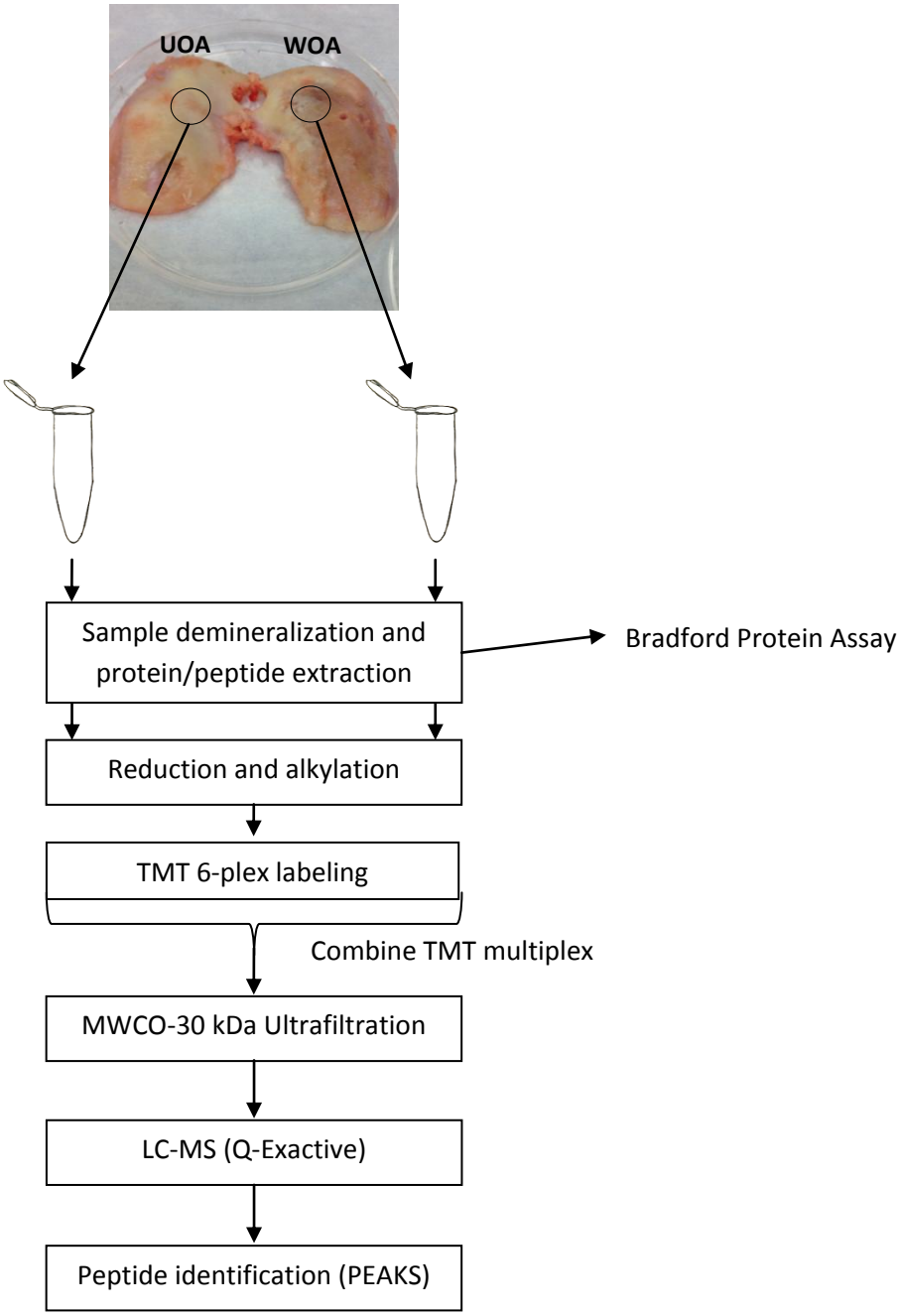


Figure 1.

LC-MS/MS Analysis

The samples were dissolved in 2% ACN, 0.1% TFA (v/v, 7 µl). Aliquots of 5 µl were loaded on a Dionex nano-LC instrument (Ultimate 3000 RSLC, Thermo Fisher Scientific, USA) fitted with a 75 µm x 2 cm trap column (PepMap Acclaim C_{18}, Thermo Fisher Scientific, USA) and a 75 µm x 50 cm separation column (PepMap Acclaim C_{18}, Thermo Fisher Scientific, USA), coupled to a Q-Exactive mass spectrometry instrument (Thermo Fisher Scientific, USA). Peptide separation was performed using a 160 min gradient running from 3 to 45 % of mobile phase B (84% ACN, 0.1% formic acid). The mass spectrometer was operated in the positive ion mode. The instrument settings for the MS scans were: resolution 70,000; *m/z* range 400-1,600; max injection time 250 ms; AGC target 1e6. Data-dependent acquisition was used to record up to 10 consecutive MS^2 spectra per full scan spectrum, selecting precursor ions in decreasing order of intensity, and using 20 s dynamic exclusion, and charge state exclusion to exclude signals with unassigned charge, charge 1 and >5. The isolation window was set to 1.2 *m/z*. The instrument settings for the MS^2 scans were: resolution 35,000 for endogenous peptides and 17,500 for tryptic peptides; fixed first mass *m/z* 100; max injection time 120 ms for endogenous peptides and 60 ms for tryptic peptides; AGC target 1e5.

Data Analysis

Automatic *de novo* sequencing and peptide identification by sequence database searching was performed using the software PEAKS Studio (Bioinformatics Solutions Inc, Canada). The following settings were used: database: UniProt/SwissProt; taxonomy: homo sapiens; parent mass error tolerance: 20 ppm; enzyme: none; fixed modifications: carbamidomethylation, TMT6-plex; variable modifications: oxidation of methionine. Peptide identifications were validated by the commonly used method target-decoy approach, using a target false discovery

rate (FDR) of 5% [20]. TMT reporter ion intensities were determined using the software Proteome Discoverer 2 (Thermo Fisher Scientific, USA). Spectral clustering was performed using MSCluster to match spectra representing the same peptide in different TMT sets and the cluster list was annotated using the peptide identification results from PEAKS [21]. TMT ion intensity ratios were normalized to the average ratio within the TMT set and log-transformed.

Protocol for efficient peptide extraction from osteochondral plug

A key to a successful analysis is efficient peptide extraction [22]. The inorganic matrix of bone with high abundance of hydroxyapatite complicates the extraction process. Previous reports have shown that protein extraction from bone tissue is significantly improved by removing the inorganic matrix before the extraction process [23, 24]. Bone samples were therefor initially demineralized by incubation in 1.2 M HCl.

The mean protein concentration obtained from the 6 patients in UOA and WOA zones of the OA samples, as determined by the Bradford protein assay were per biopsy sample, 28.3 µg (SD 17.7) from macroscopic healthy tissue (UOA), and 46.7 µg (SD 20.7) from osteoarthritic tissue (WOA).

Statistics

Data was adjusted to be normally distributed by log transformation. Data was plotted to confirm the distribution. The difference between mean values for the peptide abundance from unwounded (UOA) and wounded (WOA) zones from the 6 patients investigated was tested for statistical significance (90% significance level $p \leq 0.1$) using two tailed paired t-test. Due

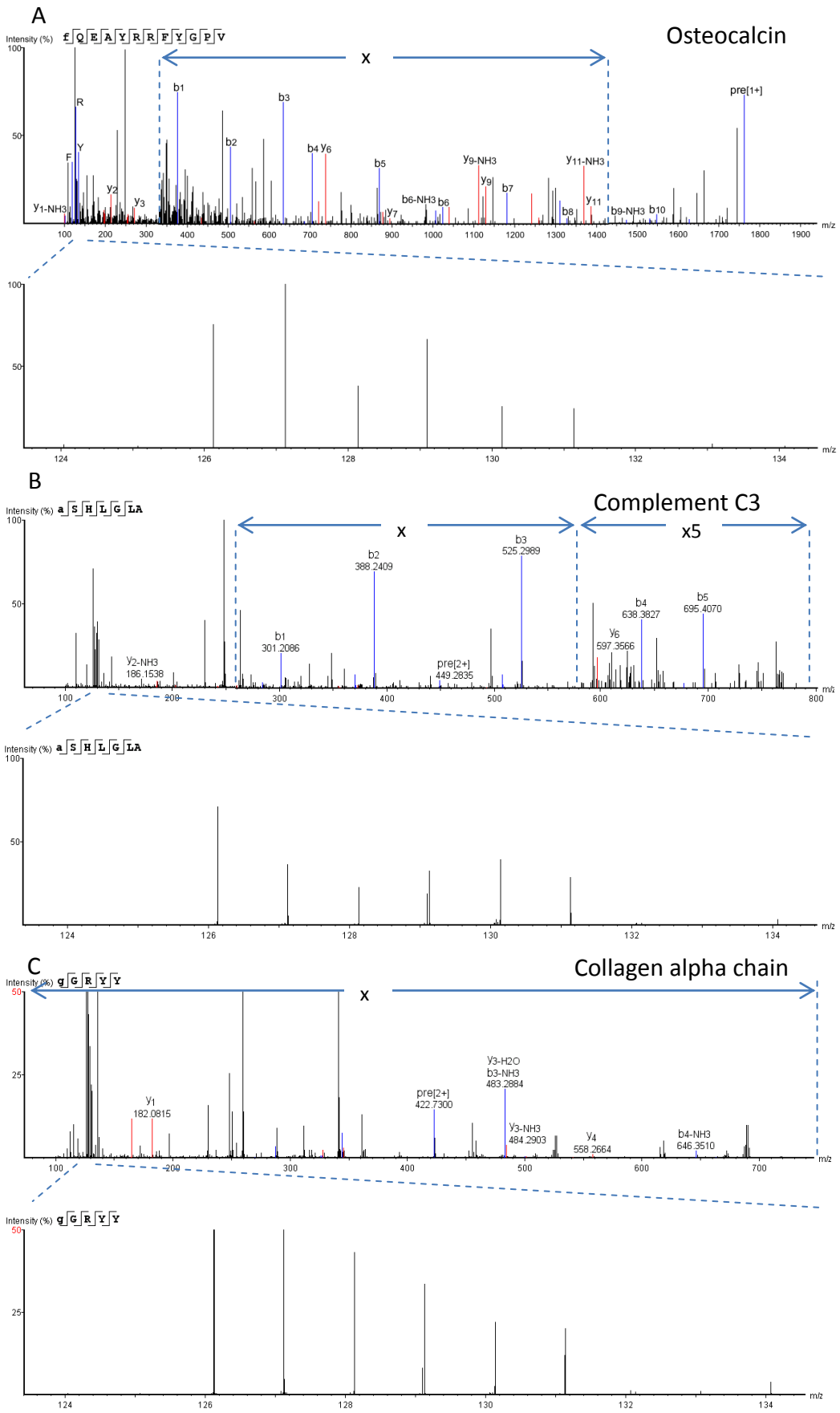
to the high dimension of data and multiple hypothesis testing multiple testing adjustment analysis with Holm's and Hochberg's method was made.

Results

Endogenous peptides identification from wounded and unwounded zones from OA samples

Extracts of endogenous peptides, prepared by molecular weight cut-off (MWCO) ultrafiltration, were analyzed by LC-MS in the data-dependent mode, and peptide identification was performed by database searching. The term endogenous peptides refer to peptides that are not obtained by tryptic digestion of the sample proteins, as is the most common types of peptides analyzed in proteomics.

In total, 6292 endogenous peptides were identified, derived from 915 proteins (889 protein groups). Out of these, 601 peptides (derived from 156 proteins) carried TMT label. 462 of the identified peptide chains were able to match with the database used for identification. A complete list of identified and matched peptides and their corresponding proteins is provided in Additional File 1.



In the six patients included in the study, a total of 566 endogenus peptides were found significantly differing with a p-value≤0.1 in unwounded zones compared to wounded osteoarthritic zones. Out of the significant differing findings, only 8 proteins and endogenous peptides were identified in the database search. A complete list of identified endogenous peptides and proteins can be found in Additional File 1. After multiple testing adjustments there was no significant difference in peptides expressed in wounded and unwounded zones.

Table 1 presents proteins identified found in 3 or more patients with a p-value≤0.1.

Discussion

This is to our knowledge the first reported peptidomic study of cartilage and subchondral bone carried out in human samples. Previous studies have investigated protein expression in synovial fluid and the synovial membrane. Although we did experience difficulties with labeling and quantification of peptides we feel that the results are important to report as peptidomics on solid tissue can potentially bring many answers to the field of OA research.

We did find a group of peptides varying significantly between the groups tested but none of these differed significantly when we did multiple testing adjustments.

In our study we identified multiple peptides related to proteins involved in neuronal signaling. None of the neuro related proteins were found to differ significantly between unwounded and wounded zones but are interesting in their presence in subchondral bone and cartilage for the deepening of our knowledge on the pain mechanisms involved in OA.

Previously reported OA associated proteins

One of the interesting findings is the expression of Complement C3. Recent findings indicate that complement cascade activation may be crucial for the development of OA [25]. Our findings indicate a difference in complement C3 in macroscopically healthy compared to wounded zones of OA tissue. Complement C3 was decreased in wounded zones compared to unwounded zones and a reason for this is probably that even though the healthy tissue was taken from unwounded zones, the biopsies came from patient with manifest OA. Complement C3 activations can be seen both in early and advanced stages of OA but is predominantly thought to play an important role in early stages of osteoarthritis development [26]. Knockout mice for C3 have however not shown to be protected against the development of OA and the reason for this is that compensatory mechanisms with coagulations factors activate C5 leading to a complement cascade activation even though C3 is not present [27].

Changes to the subchondral bone may be of equal importance to the pathogenesis of OA. Recent studies suggest that a decrease in the subchondral bone density may be related to OA [28]. Moreover, knockout mice models with defect type I collagen in bone show progressive cartilage destruction [29]. In our biopsies both cartilage and subchondral bone was present. We found a decrease in collagen I in the wounded zones correlating with previous findings that changes in the subchondral bone may be related to disease progression in OA.

With a progressive OA there is also evidence of increased bone turnover and metabolic activity in the subchondral bone [30-32]. Osteocalcin is the most abundant noncollagenous protein found in bone and plays a major role in bone formation. Previous studies have suggested an initial decrease in Osteocalcin in early OA followed by an increase as the disease progresses.

In early stages of rheumatoid arthritis, plasma components such as fibrinogen aggregate in the joint and possibly initiate the inflammatory cascade. Excessive fibrin deposits in the synovial membrane are thought to play a role in pannus formation and the disease progression [33, 34]. Activation of the inflammation cascade can also be seen in OA and fibrinogen may play an equally important role in early stages of the disease. In our data we saw decreased levels of fibrinogen in the wounded tissue compared to the unwounded zones present in 5 out of 6 patients.

Neuropeptide signaling pathways in OA

Multiple endogenous peptides involved in neurotransmitter signaling were identified although none of them differed significantly between the unwounded and wounded areas. The findings were however interesting in our quest for understanding the joint pain mechanisms better.

An endogenous peptide sequence derived from VPS10 domain-containing receptor SorCS2-receptor was present in all 6 patients investigated. Seen in murine models, SorCS2 predominantly plays an important role in the development of the central nervous system [35]. SorCS2 has recently been associated with crucial ligament rupture and post-traumatic osteoarthritis in animal models [36, 37] and is linked to activation of neuropeptide pathways [38].

Vesicular acetylcholine transporter (SLC18A3) is responsible for the acetylcholine transportation into synaptic vesicles and subsequently crucial for acetylcholine secretion [39]. Peptide sequences from SLC18A3 were present in all 6 patients. Previous studies have shown that various choline and acetylcholine transporters are expressed in synovial tissue and cartilage from patients with rheumatoid arthritis and OA however SLC18A3 was absent [40].

SH3 and multiple ankyrin repeat domains protein 2 (SHANK2) is involved in synaptic plasticity through its regulation of NMDA receptors as well as regulating the spine [41]. SHANK2 endogenous peptide sequences were present in all 6 patients. Animal models indicate SHANK2 may play an important role in chronic pain mechanism, knockout mice have shown decreased sensitivity to chronic pain [42].

4.3 Analytical considerations and limitations of the study

We had only a small amount of material, investigating osteochondral plugs from 6 patients undergoing total knee replacement. To be able to identify clinical relevant significant differences, with so many peptides identified, a larger group of patients is needed. Moreover, a vast majority of the significant peptides were not identified in the database search. With increasing peptidomic research we hope the database is expanding which in the future can lead to a better peptide identification process.

The TMT labeling was found to be incomplete, which resulted in that quantitative data could only be obtained for a fraction of all identified peptides. We did following up series with other control tissue that showed that the TMT markers were very thermal sensitive and that they needed to be adjusted to room temperature a couple of hours for a better labelling. While it may distort the quantitative results, we still feel these results are worth reporting.

The present pilot study shows that the TMT multiplex LC-MS/MS method is useful for identification of endogenous peptides in cartilage and subchondral bone from OA patients. With this new method it is possible to analyze endogenous peptides in tissue directly taken from surgical procedures without any cell culturing needed. The findings open up for further

studies potentially deepening our understanding of the development of pain and the pathogenesis of OA.

Contribution,

HZ, KB and MB designed the study. JG and BG performed the experimental work and with TS analyzed the data. JG performed the MS analysis. HZ, MB, KB and JG critically evaluated the study. BG drafted the manuscript, JG and MB finalized the article. All authors discussed on the work and gave final approval.

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Ethics approval

Tissue sampling was approved by patients and followed a procedure approved by the Ethics Committee at University of Gothenburg. Since tissue samples were de-identified the need for an ethical approval was waived.

Conflict of Interest statement

The authors have declared no conflict of interest.

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Legends

Figure1: Schematic workflow of the steps in analysis of cartilage and subchondral biopisis from OA patients using peptidomics. Samples from 6 patients were included in the study. 3 biopsies were taken from wounded(WOA) and unwounded(UOA) zones. Peptides were extracted and iTRAQ labeled. Labeled samples were then fractioned using ultrafiltration and analysed on a LC-MS (Q-Exactive).

Figure 2. Identification and relative quantification of endogenous peptides. Fragment ion mass spectrum of identified endogenous peptides (a) Osteocalcin 89-100, (b) Complement C3 742-747, and (c) Collagen alpha chain 1212-1216. The spectra are annotated with matching b- and y-ions. The six TMT reporter ions of m/z 127-131 in the lower mass region give the relative abundance of the peptides in osteoarthritis (WOA) tissue and tissue from unwounded zones (UOA) from a single patient.

Tables

Table 1

Gene	Identification Uniprot	Peptide sequence	Present in # patients	Sample mean relative difference (WOA-UOA)	p-value
HBB	Hemoglobin subunit beta	HLTPE	6	-0.5	0.009
C3	Complement C3	ASHLGLA	5	-0.6	0.009
COL1A1	Collagen alpha-1(I) chain	GGRYY	6	-0.5	0.026
BGLAP	Osteocalcin	EAYRRFYGPV	5	-0.5	0.046
COL1A2	Collagen alpha-2(I) chain	GGGYDFGYDGDFY	3	-0.4	0.069
FGA	Fibrinogen alpha chain	PGFFSPMLGEFVSETESRG	5	-0.3	0.073
COL1A1	Collagen alpha-1(I) chain	HDGGRYYR	6	-0.3	0.082
FGA	Fibrinogen alpha chain	RHPDEAAF	4	-0.4	0.096

Additional file 1. List of endogenous peptides differing in unwounded(UOA) and wounded zones (WOA) from cartilage and subchondral bone in OA patients.

N	Gene	Protein name	Peptide sequence	p-value
1	HBB	Hemoglobin subunit beta	HLTPE	0.009
2	C3	Complement C3	ASHLGLA	0.009
3	COL1A1	Collagen alpha-1(I) chain	GGRYY	0.026
4	BGLAP	Osteocalcin	EAYRRFYGPV	0.046
5	COL1A2	Collagen alpha-2(I) chain	GGGYDFGYDGDFY	0.069
6	FGA	Fibrinogen alpha chain	PGFFSPMLGEFVSETESRG	0.073
7	COL1A1	Collagen alpha-1(I) chain	HDGGRYYR	0.082
8	FGA	Fibrinogen alpha chain	RHPDEAAF	0.096
9	VIM	Vimentin	ALRPST	0.102
10	F2	Prothrombin	DRAIEGRTAT	0.105
11	BGLAP	Osteocalcin	GFQEAYRRFYGPV	0.132
12	RNASE1	Ribonuclease pancreatic	PYVPVHFDASVEDST	0.142
13	HBA1	Hemoglobin subunit alpha	GAHAGEYGAEALER	0.152
14	LYZ	Lysozyme C	TDYGIFQIN	0.159
15	PLA2G2A	Phospholipase A2, membrane associated	KFLSYKFSN	0.188
16	VIM	Vimentin	LGSALRPSTSRS	0.189
17	ZNF106	Zink finger protein	DFSLITTGIQEPQ	0.201
18	COL1A1	Collagen alpha-1(I) chain	YGYDE	0.216
19	PLA2G2A	Phospholipase A2, membrane associated	LSYKFS	0.221
20	EIF3F	Eukaryotic translation initiation factor 3 subunit F	PAQTPAPALP	0.224
21	VIM	Vimentin	ALRPSTS	0.229
22	AHSG	Alpha-2-HS-glycoprotein	GEVSHPRK	0.232
23	HBA1	Hemoglobin subunit alpha	YFPHFDLSH	0.235
24	C3	Complement C3	HLGLA	0.250
25	COL3A1	Collagen alpha-1(III) chain	VGAAAIAGIG	0.258
26	ALB	Serum albumin	AHKSEVAHR	0.265
27	PLA2G2A	Phospholipase A2, membrane associated	LVNFHRMIKL	0.268
28	FREM2	FRAS1-related extracellular matrix protein 2	PEILNTGFT	0.270
29	C3	Complement C3	YITELRRQHARASHLGLA	0.274
30	VIM	Vimentin	LGSALRPSTSR	0.289
31	AHSG	Alpha-2-HS-glycoprotein	GEVSHPRKT	0.289
32	VIM	Vimentin	RPSSSRSYVTT	0.294
33	CILP	Cartilage intermediate layer protein 1	VQGRVPSRRQQ	0.304
34	MAP3K1	Mitogen-activated protein kinase kinase kinase 1	VGPSSSTTTTTTT	0.312
35	SPP1	Osteopontin	RGDSVVYGLR	0.313
36	SASH1	SAM and SH3 domain-containing protein 1	EPPPQIVPEVPQ	0.328
37	SERPINE3	Serpin E3	ISPAGVS	0.335
38	SPP1	Osteopontin	KQAD	0.339
39	PLA2G2A	Phospholipase A2, membrane associated	LVNFH	0.343
40	AHSG	Alpha-2-HS-glycoprotein	PPGHQLHR	0.348
41	SPP1	Osteopontin	VVYGLR	0.356
42	VIM	Vimentin	LRPSTSRSLY	0.359
43	PLA2G2A	Phospholipase A2, membrane associated	SYGFYG	0.379
44	LRRN2	Leucine-rich repeat neuronal protein 2	QPRKGVGGRRPLPP	0.383
45	FGA	Fibrinogen alpha chain	SSSHHPGIAEFPSRG	0.384
46	COL5A2	Collagen alpha-2(V) chain	GEAGPEGPPGEPG	0.385
47	COL5A2	Collagen alpha-2(V) chain	FPGSAGR	0.385
48	PLA2G2A	Phospholipase A2, membrane associated	PKDA	0.388
49	SPP1	Osteopontin	LAPQNAV	0.390
50	VIM	Vimentin	PGGVYATRSS	0.399
51	HBB	Hemoglobin subunit beta	VVYPWTQRF	0.400
52	COL1A1	Collagen alpha-1(I) chain	AGFDF	0.400
53	BGLAP	Osteocalcin	DELADHIGFQEAYRRFYGP	0.402
54	COL2A1	Collagen alpha-1(II) chain	PDPLQYMRA	0.407
55	SORCS2	VPS10 domain-containing receptor SorCS2	PGAPGPSPG	0.407
56	VIM	Vimentin	RPSTSRSLY	0.408
57	SPP1	Osteopontin	GDSVVYGLR	0.417
58	TFA4	Transcription initiation factor TFIID subunit 4	RSPGVQPQLV	0.419
59	DONSON	Protein downstream neighbor of Son	SRGAAASPP	0.423
60	HBA1	Hemoglobin subunit alpha	PHFDLSHGSAQ	0.425
61	PLA2G2A	Phospholipase A2, membrane associated	LVNFHRMIKLTTG	0.425
62	VIM	Vimentin	GVYATRSSAVR	0.433
63	RAB3A	Ras-related protein Rab-3A	DHLGFEFFEASAKDN	0.435
64	FGB	Fibrinogen beta chain	EEAPSLRPAPPPISGGGY	0.439

65	HBA1	Hemoglobin subunit alpha	HGKK	0.441
66	HBA1	Hemoglobin subunit alpha	FPHFDLSHG	0.441
67	COL4A3	Collagen alpha-3(IV) chain	DEGTPGPPG	0.450
68	VIM	Vimentin	GPGTASRP	0.451
69	PLA2G2A	Phospholipase A2, membrane associated	EAALSY	0.460
70	APOA2	Apolipoprotein A-II	VELGTQPATQ	0.464
71	VIM	Vimentin	LPLVDTHSKRTL	0.467
72	ANG	Angiogenin	LPVHLDQSIFRRP	0.468
73	COL8A2	Collagen alpha-2(VIII) chain	LPGPPGEGRAGE	0.470
74	COL7A1	Collagen alpha-1(VII) chain	PGEGGIAPGEPGL	0.480
75	COL1A2	Collagen alpha-2(I) chain	FYRA	0.482
76	FGB	Fibrinogen beta chain	REEAPSLRPAPPPISGGGY	0.486
77	PLA2G2A	Phospholipase A2, membrane associated	FLSYKFSNSG	0.487
78	VIM	Vimentin	SSPGGVYATRSSAVR	0.488
79	PML	Protein PML	GLLEGPALARA	0.490
80	VIM	Vimentin	SALRPSTS	0.493
81	VIM	Vimentin	LYASSPGGVYAT	0.497
82	VIM	Vimentin	ETRDGQVINETSQHHDDLE	0.505
83	VIM	Vimentin	VTTSTR	0.508
84	DNAH12	Dynein heavy chain 12, axonemal	IPFEAISY	0.508
85	COL1A1	Collagen alpha-1(I) chain	DGGRYY	0.511
86	PLA2G2A	Phospholipase A2, membrane associated	ALSYGFY	0.514
87	VIM	Vimentin	LPLVDTH	0.516
88	VIM	Vimentin	PSSSRSYVTT	0.518
89	AHSG	Alpha-2-HS-glycoprotein	VLLAAPPG	0.519
90	BGLAP	Osteocalcin	IGFQEAYRRFYGPV	0.525
91	HBA1	Hemoglobin subunit alpha	LSPADKTNVK	0.528
92	COL1A1	Collagen alpha-1(I) chain	TGGISVPG	0.528
93	PLA2G2A	Phospholipase A2, membrane associated	YQYY	0.529
94	AHSG	Alpha-2-HS-glycoprotein	EVSHPRK	0.531
95	VIM	Vimentin	YVTTSTRTYSLG	0.534
96	C2orf40	Augurin	PYGFRHGA	0.535
97	COL1A1	Collagen alpha-1(I) chain	EQGVPGDLGAPG	0.536
98	VIM	Vimentin	GVYATRSSAVRL	0.541
99	BGLAP	Osteocalcin	YRRFYGPV	0.545
100	COL1A1	Collagen alpha-1(I) chain	EPGPTGLPGPPGE	0.555
101	MGP	Matrix Gla protein	MESYELN	0.557
102	COL1A2	Collagen alpha-2(I) chain	TVGPAGIRG	0.558
103	COL1A2	Collagen alpha-2(I) chain	GVVGPTGPVGAAGPAGP	0.559
104	F2	Prothrombin	TATSEYQTFFNPRTFG	0.569
105	FAT1	Protocadherin Fat 1	DIRSNAEIT	0.570
106	ALB	Serum albumin	FQNAL	0.575
107	COL1A2	Collagen alpha-2(I) chain	GYDFGYDGDFYRA	0.575
108	HBB	Hemoglobin subunit beta	LTPEEK	0.576
109	PTPN13	Tyrosine-protein phosphatase non-receptor type 13	CSQPALT	0.577
110	COL1A1	Collagen alpha-1(I) chain	ISVPGPM	0.577
111	HBA1	Hemoglobin subunit alpha	SKYR	0.578
112	VIM	Vimentin	SALRPSTSR	0.578
113	VIM	Vimentin	ETRDGQVINETSQ	0.587
114	COL4A4	Collagen alpha-4(IV) chain	GPIGLSG	0.587
115	COL5A1	Collagen alpha-1(V) chain	GAPGADGPQGPPGGIG	0.588
116	COL1A2	Collagen alpha-2(I) chain	GYDFGY	0.592
117	SPARC	SPARC	AEETEEEVVAEN	0.593
118	COL1A2	Collagen alpha-2(I) chain	FGYDGDFYR	0.597
119	VIM	Vimentin	TVETRDGQVINETSQHHDDLE	0.599
120	COL8A1	Collagen alpha-1(VIII) chain	RGMGGVPGALG	0.602
121	VIM	Vimentin	GPGTASRPSSSRSYVTTST	0.606
122	ZFHX2	Zinc finger homeobox protein 2	LSPPP	0.608
123	COL1A1	Collagen alpha-1(I) chain	YDEKSTG	0.611
124	VIM	Vimentin	LGSALRPS	0.614
125	COL1A2	Collagen alpha-2(I) chain	GHPGTVGPAGIRGPQ	0.615
126	LRRN2	Leucine-rich repeat neuronal protein 2	QPRKGVGGRRPLPPA	0.616
127	VIM	Vimentin	ALRPSTSRSLY	0.617
128	LRRC3C	Leucine-rich repeat-containing protein 3C	SGWGGARRSTDVALLVT	0.618
129	PLA2G2A	Phospholipase A2, membrane associated	EAALSYGF	0.621
130	VIM	Vimentin	TNLDSLPLVD	0.622
131	VIM	Vimentin	GGPGTASRPSSSRSYVTT	0.627
132	COL1A2	Collagen alpha-2(I) chain	LRGEIGN	0.627
133	COL1A2	Collagen alpha-2(I) chain	VGPAGPRGPAGPSG	0.629
134	ANG	Angiogenin	IFRRP	0.630
135	COL2A1	Collagen alpha-1(II) chain	PDPLQY	0.631
136	BGN	Biglycan	VLDPD	0.633
137	CADPS2	Calcium-dependent secretion activator 2	DMDTALEAQPQD	0.634

138	RNASE1	Ribonuclease pancreatic	PYVPVHFDASVE	0.635
139	BGN	Biglycan	DTSGVLDPD	0.635
140	HBA1	Hemoglobin subunit alpha	GAHAGEYGAEALE	0.637
141	FGA	Fibrinogen alpha chain	LAEGGGVR	0.641
142	FAM160A2	FTS and Hook-interacting protein	HASWARGP	0.643
143	PLIN4	Perilipin-4	EERDAGVLSRVCGLL	0.643
144	CASZ1	Zinc finger protein castor homolog 1	AAGAGARTPAL	0.648
145	COL4A3	Collagen alpha-3(IV) chain	GSPGLPGSPGPPG	0.652
146	VIM	Vimentin	RSSAVRLR	0.655
147	VIM	Vimentin	GPGTASRPSSSRSYVTT	0.656
148	VIM	Vimentin	VTTSTRTY	0.658
149	VIM	Vimentin	TNLDSLPLVDTH	0.664
150	FGB	Fibrinogen beta chain	REEAPSLRPAPPPISGGGYR	0.666
151	KIF14	Kinesin-like protein KIF14	GIDGSK	0.674
152	COL1A1	Collagen alpha-1(I) chain	ISVPGPMGP	0.681
153	ALB	Serum albumin	VAASQAALGL	0.685
154	COL8A1	Collagen alpha-1(VIII) chain	DRGMGGVPGALG	0.685
155	VIM	Vimentin	LNLRETNLD	0.687
156	VIM	Vimentin	ALRPSTSR	0.689
157	VIM	Vimentin	PSTSRSLY	0.689
158	CRNKL1	Crooked neck-like protein 1	EEEVKANPHN	0.690
159	COL2A1	Collagen alpha-1(II) chain	PIGPPGERG	0.691
160	COL24A1	Collagen alpha-1(XXIV) chain	MGYPGPPGV	0.691
161	VIM	Vimentin	ALRPSTSRSLYA	0.694
162	COL1A1	Collagen alpha-1(I) chain	DGGRYYRA	0.697
163	VIM	Vimentin	VPGVRL	0.705
164	COL1A2	Collagen alpha-2(I) chain	GGYDFGYDGDFYR	0.705
165	COL1A2	Collagen alpha-2(I) chain	QGAPGSVGPAGPRGPAGPSGPAG	0.707
166	COL1A1	Collagen alpha-1(I) chain	PAGPRGPPG	0.707
167	COL2A1	Collagen alpha-1(II) chain	PGPAGPQGAPGPA	0.708
168	VIM	Vimentin	PGGVYATRSSA	0.709
169	HBA1	Hemoglobin subunit alpha	FPHFDL	0.712
170	PLA2G2A	Phospholipase A2, membrane associated	KEAAL	0.712
171	VIM	Vimentin	TRTYSLG	0.714
172	BGLAP	Osteocalcin	LYQWLG	0.715
173	BAIAP3	BAI1-associated protein 3	PDPAQAQGLGT	0.717
174	VIM	Vimentin	LADAIN	0.717
175	COL11A2	Collagen alpha-2(XI) chain	PSGPPGPAGSPG	0.717
176	WWC1	Protein KIBRA	LDLELDLQAT	0.719
177	COL1A2	Collagen alpha-2(I) chain	DGDFYRA	0.719
178	BGLAP	Osteocalcin	ELADHIGFQEAYRRFYGPV	0.719
179	COL1A2	Collagen alpha-2(I) chain	DFGYDGDFYRA	0.722
180	COL3A1	Collagen alpha-1(III) chain	GMRGMPGSPGGP	0.723
181	ITIH4	Inter-alpha-trypsin inhibitor heavy chain H4	AHIRFK	0.723
182	HBA1	Hemoglobin subunit alpha	AGEYGAEALER	0.723
183	COL1A2	Collagen alpha-2(I) chain	YDFGYDGDFYRA	0.724
184	ANKRD13C	Ankyrin repeat domain-containing protein 13C	PGDEEAAAALGGTFT	0.725
185	VIM	Vimentin	GPGTASRPSSSRSYVTTSTRTYS	0.727
186	SMARCA1	Probable global transcription activator SNF2L1	DYCMWRGYE	0.728
187	PLA2G2A	Phospholipase A2, membrane associated	YKFSN	0.733
188	IGFN1	Immunoglobulin-like and fibronectin type III domain-containing protein 1	PVAGLSDSG	
189	HECA	Headcase protein homolog	YGARSPGGSPGQSPPTG	0.734
190	VIM	Vimentin	GGPGTASRPSSS	0.734
191	COL5A2	Collagen alpha-2(V) chain	PPGSAGPPGSPGPQ	0.734
192	AATK	Serine/threonine-protein kinase LMTK1	SRFTVSPAPT	0.735
193	VIM	Vimentin	YVTTSTRTY	0.737
194	FGA	Fibrinogen alpha chain	SGEGDFLAEGGGVR	0.741
195	COL1A2	Collagen alpha-2(I) chain	YDGDFYR	0.744
196	FGA	Fibrinogen alpha chain	VPGNF	0.745
197	SLC22A31	Putative solute carrier family 22 member 31	LVLTTGLGAS	0.746
198	COL1A2	Collagen alpha-2(I) chain	GYDGDFY	0.746
199	ANG	Angiogenin	RNVVVACENGLPVHLDQSIFRRP	0.747
200	VIM	Vimentin	VETRDGQVINETSQHHDDLE	0.747
201	WIZ	Protein Wiz	QDAGLHLDLPAQ	0.749
202	VIM	Vimentin	TRSSAVRLR	0.752
203	COL5A2	Collagen alpha-2(V) chain	LPEFTE	0.755
204	BGLAP	Osteocalcin	QEAYRRFYGPV	0.755
205	SLC18A3	Vesicular acetylcholine transporter	ISFGSLVA	0.758
206	NCOR1	Nuclear receptor corepressor 1	QSDNDSSATCSADED	0.758
207	DIAPH3	Protein diaphanous homolog 3	DLHDKFVT	0.760

208	VIM	Vimentin	QVINETSQHHDDLE	0.760
209	VIM	Vimentin	VPGVR	0.760
210	HBA1	Hemoglobin subunit alpha	YFPHFDLSHGSAQV	0.760
211	COL1A1	Collagen alpha-1(I) chain	QGVPGDLGAPG	0.760
212	SHANK2	SH3 and multiple ankyrin repeat domains protein 2	PSPALSDVFSL	0.762
213	COL1A2	Collagen alpha-2(I) chain	VGPVGPAGPIG	0.762
214	PLA2G2A	Phospholipase A2, membrane associated	TGKEAAL	0.762
215	CPEB3	Cytoplasmic polyadenylation element-binding protein 3	SFRRFG	0.762
216	FGA	Fibrinogen alpha chain	DFLAEGGGVR	0.764
217	COL1A2	Collagen alpha-2(I) chain	FGYDGDFYRA	0.764
218	GLTSCR1	Glioma tumor suppressor candidate region gene 1 protein	PAPTAPGPPQ	0.766
219	COL2A1	Collagen alpha-1(II) chain	PLQYM	0.767
220	COL5A2	Collagen alpha-2(V) chain	MPDPLPEFTE	0.769
221	SPP1	Osteopontin	KQADSG	0.771
222	PLEKHO1	Pleckstrin homology domain-containing family O member 1	LLET	0.771
223	FRMPD3	FERM and PDZ domain-containing protein 3	SSSPEASRTQE	0.771
224	COL1A1	Collagen alpha-1(I) chain	FDFSFLPQ	0.773
225	FGA	Fibrinogen alpha chain	PDLVPGNF	0.775
226	RNASE1	Ribonuclease pancreatic	PYVPVHFD	0.776
227	COL1A2	Collagen alpha-2(I) chain	GYDGDFYRA	0.777
228	BGN	Biglycan	LDPDSVTPTY	0.777
229	AHSG	Alpha-2-HS-glycoprotein	GPGLIYRQPN	0.779
230	COL2A1	Collagen alpha-1(II) chain	PPGSPGPAGP	0.779
231	VIM	Vimentin	ALRPSTSRS	0.779
232	VIM	Vimentin	SAVRLR	0.781
233	HBA1	Hemoglobin subunit alpha	LSFPTTK	0.781
234	HBA1	Hemoglobin subunit alpha	YFPHFDLS	0.782
235	HBA1	Hemoglobin subunit alpha	YFPHFDLSHG	0.784
236	SPP1	Osteopontin	ELDSASSEVN	0.784
237	COL1A2	Collagen alpha-2(I) chain	DGDFYR	0.786
238	RNF175	RING finger protein 175	IVELDEEGLIEN	0.787
239	HBB	Hemoglobin subunit beta	HLTPEEK	0.787
240	COL4A3	Collagen alpha-3(IV) chain	HLGPAGPEGAPG	0.788
241	PLA2G2A	Phospholipase A2, membrane associated	LVNFHRM	0.790
242	VIM	Vimentin	PGGVYATRSSAVR	0.790
243	COL5A2	Collagen alpha-2(V) chain	PTGAVGFAGPQGPDGQ	0.790
244	VIM	Vimentin	SVPGVRLLQ	0.791
245	AHSG	Alpha-2-HS-glycoprotein	VVQPSVGAAAG	0.791
246	C3	Complement C3	HARASHLGLA	0.795
247	IRF7	Interferon regulatory factor 7	GGPGLPAGELYGWAVETT	0.800
248	COL16A1	Collagen alpha-1(XVI) chain	NNGLPGQP	0.800
249	COL1A2	Collagen alpha-2(I) chain	GGYDFGYDGDFYRA	0.802
250	NCOA6	Nuclear receptor coactivator 6	NQLPSGALQ	0.804
251	COL1A1	Collagen alpha-1(I) chain	GRYYRA	0.804
252	AHSG	Alpha-2-HS-glycoprotein	PPDSHVLLAAPPG	0.809
253	RNASE1	Ribonuclease pancreatic	VPVHFDASVEDST	0.810
254	VIM	Vimentin	SLNLRETNLD	0.812
255	SPP1	Osteopontin	RGDSVVYG	0.813
256	RNASE1	Ribonuclease pancreatic	PYVPVH	0.814
257	**TNR**	**Tenascin-R**	**VFQRRQ**	**0.818**
258	VIM	Vimentin	GGPGTASRPSSSR	0.819
259	VIM	Vimentin	LGSALRPSTSRSLY	0.819
260	COL5A2	Collagen alpha-2(V) chain	YDESMPDPLPEFTE	0.819
261	VIM	Vimentin	AVRLR	0.821
262	PKDREJ	Polycystic kidney disease and receptor for egg jelly-related protein	VYDSLGAFSQVT	0.822
263	MPL	Thrombopoietin receptor	QYLRDTAALSP	0.822
264	SAP30	Histone deacetylase complex subunit SAP30	VPGAGAVSAAGP	0.824
265	VIM	Vimentin	GVYATRSSA	0.824
266	HBA1	Hemoglobin subunit alpha	YFPHFDL	0.825
	HBA1	Hemoglobin subunit alpha	AGEYGAEAL	0.826
267	PLA2G2A	Phospholipase A2, membrane associated	ALSYGFYG	0.827
268	PLEKHO1	Pleckstrin homology domain-containing family O member 1	PSPEEPT	0.827
269	COL1A2	Collagen alpha-2(I) chain	QGAPGSVGPAGPRGPAGPSGPA	0.828
270	COL1A2	Collagen alpha-2(I) chain	GDFYRA	0.828
271	COL1A2	Collagen alpha-2(I) chain	GYDFGYDGD	0.829
272	COL11A2	Collagen alpha-2(XI) chain	ERGAAGSGGPIGPPGRPGPQGPPGA	0.829
273	COL1A2	Collagen alpha-2(I) chain	ERGEVGPAGPN	0.830

274	PLA2G2A	Phospholipase A2, membrane associated	LVNFHR	0.830
275	KAT6B	Histone acetyltransferase KAT6B	EAVPESDEE	0.830
276	MSLNL	Mesothelin-like protein	RPYLGGSPGGAQP	0.830
277	C3	Complement C3	ARASHLGLA	0.831
278	VIM	Vimentin	VTTSTRTYSLG	0.832
279	COL1A1	Collagen alpha-1(I) chain	ISVPGPMGPS	0.832
280	COL1A2	Collagen alpha-2(I) chain	DFGYDGDFY	0.833
282	COL1A1	Collagen alpha-1(I) chain	PPSAGFDF	0.834
283	COL22A1	Collagen alpha-1(XXII) chain	AGPPGLPGTTSLFT	0.839
284	FGA	Fibrinogen alpha chain	GEGDFLAEGGGVR	0.840
285	AHSG	Alpha-2-HS-glycoprotein	VVQPSVG	0.842
286	ALB	Serum albumin	VTDLTK	0.842
287	PLA2G2A	Phospholipase A2, membrane associated	AALSYGFY	0.842
288	R3HDM1	R3H domain-containing protein 1	SVIPPG	0.843
289	COL1A2	Collagen alpha-2(I) chain	GGYDFGYDGDFY	0.846
290	SPP1	Osteopontin	DSVVYGLR	0.846
291	COL1A1	Collagen alpha-1(I) chain	SYGYDE	0.846
292	VIM	Vimentin	LYASSPGGVYATR	0.846
293	SPATA31A3	Spermatogenesis-associated protein 31A3	LSPAFPSLIQ	0.850
294	VIM	Vimentin	SALRPSTSRS	0.851
295	COL1A2	Collagen alpha-2(I) chain	AGPAGPAGPRG	0.851
296	COL1A1	Collagen alpha-1(I) chain	NDGAKGDAGAPGAPG	0.851
297	VIM	Vimentin	SLNLR	0.852
298	SDK1	Protein sidekick-1	DFHGV	0.852
299	VIM	Vimentin	VDFSLADAIN	0.854
300	BGLAP	Osteocalcin	HIGFQEAYRRFYGPV	0.855
301	SPP1	Osteopontin	EDMLVVD	0.855
302	CLU	Clusterin	ITVTVPVEV	0.857
303	SLC23A3	Solute carrier family 23 member 3	LLGSPGH	0.857
304	MGP	Matrix Gla protein	YELN	0.858
305	COL1A2	Collagen alpha-2(I) chain	DFYRA	0.858
306	COL1A2	Collagen alpha-2(I) chain	DGDFY	0.859
307	PTPRJ	Receptor-type tyrosine-protein phosphatase eta	NGTGTPQVETNT	0.860
308	COL1A1	Collagen alpha-1(I) chain	YGYDEK	0.862
309	HIVEP2	Transcription factor HIVEP2	ANFQGSNPVLLEAP	0.864
310	VIM	Vimentin	ALRPSTSRSLYASSPGGVYATRSSAVRLR	0.864
311	COL1A2	Collagen alpha-2(I) chain	PVGPAGPIG	0.864
312	VTN	Vitronectin	KTYLFKGSQ	0.865
313	VIM	Vimentin	SPGGVYATRSSAVRLR	0.867
314	COL12A1	Collagen alpha-1(XII) chain	DSVGPPGPPGPAGGPG	0.867
315	VIM	Vimentin	GGPGTASRPSS	0.867
316	VIM	Vimentin	YVTTSTR	0.871
317	VIM	Vimentin	SPGGVYATRSS	0.873
318	AATK	Serine/threonine-protein kinase LMTK1	SRFTVSPA	0.874
319	VIM	Vimentin	VSSSSYR	0.874
320	COL2A1	Collagen alpha-1(II) chain	GPSGDQGASGPAGP	0.874
321	VIM	Vimentin	ETRDGQVINETSQHH	0.875
322	COL1A2	Collagen alpha-2(I) chain	YDFGYDGD	0.875
323	C2orf40	Augurin	PYGFRHGASVNYDDY	0.876
324	COL1A2	Collagen alpha-2(I) chain	GYDFGYDGDFYR	0.876
325	TSPYL6	Testis-specific Y-encoded-like protein 6	SLPESPHSPATLDYALEDPHQ	0.877
326	COL1A1	Collagen alpha-1(I) chain	FLPQPPQE	0.880
327	COL1A2	Collagen alpha-2(I) chain	YDFGYDGDFYR	0.881
328	COL1A2	Collagen alpha-2(I) chain	YDFGYDGDF	0.882
329	VIM	Vimentin	SLNLRETNLDSLPLVDTH	0.882
330	COL3A1	Collagen alpha-1(III) chain	GGFAPYYG	0.883
331	COL1A1	Collagen alpha-1(I) chain	VAGPKGPAGERG	0.888
332	COL7A1	Collagen alpha-1(VII) chain	HVAGVDGPPA	0.888
333	VIM	Vimentin	SLNLRETNLDSLPLVDTHSKRTL	0.888
334	FASN	Fatty acid synthase	LAAVNLDSSLADL	0.889
335	VIM	Vimentin	VINETSQHHDDLE	0.889
336	COL2A1	Collagen alpha-1(II) chain	PLQYMRA	0.889
337	COL16A1	Collagen alpha-1(XVI) chain	SGSMGPVGPPGP	0.891
338	BGLAP	Osteocalcin	FYGPV	0.891
339	VIM	Vimentin	QVINETSQHHD	0.891
340	VIM	Vimentin	GGPGTASRPSSSRS	0.894
341	VIM	Vimentin	LGSALRPST	0.894
342	APOA2	Apolipoprotein A-II	ELGTQPATQ	0.894
343	SPOCK2	Testican-2	LELTGTRT	0.895
344	RNASE1	Ribonuclease pancreatic	PYVPVHFDA	0.895
345	VIM	Vimentin	DSVDFSLADAIN	0.895
346	TEP1	Telomerase protein component 1	RLPGPWDS	0.895
347	COL1A2	Collagen alpha-2(I) chain	VGLGPGPM	0.896

348	RNASE1	Ribonuclease pancreatic	SPYVPVHFDA	0.900
349	COL1A1	Collagen alpha-1(I) chain	RGETGPAGPPGA	0.900
350	VIM	Vimentin	TSRSLY	0.901
351	COL5A2	Collagen alpha-2(V) chain	PGTPGPVGAPGDAGQ	0.902
352	AHSG	Alpha-2-HS-glycoprotein	SHPRKT	0.903
353	RNASE1	Ribonuclease pancreatic	PYVPVHFDASVED	0.904
354	AHSG	Alpha-2-HS-glycoprotein	VVQPSVGAAA	0.904
355	COL1A2	Collagen alpha-2(I) chain	DFGYDGDF	0.905
356	COL1A1	Collagen alpha-1(I) chain	PAGPKG	0.906
357	BGLAP	Osteocalcin	AYRRFYGPV	0.906
358	BSN	Protein bassoon	HHVLQ	0.906
359	VIM	Vimentin	SPGGVYATR	0.907
360	COL22A1	Collagen alpha-1(XXII) chain	GRAGPMGEPGRPG	0.908
361	VIM	Vimentin	SSPGGVYATRSSAVRLR	0.910
362	PLA2G2A	Phospholipase A2, membrane associated	LSYKFSNSGSR	0.911
363	C3	Complement C3	CNYITELRRQHARASHLGLA	0.911
364	PLA2G2A	Phospholipase A2, membrane associated	TKFL	0.911
365	RUSC2	Iporin	FLSAAHTVC	0.914
366	CNPY3	Protein canopy homolog 3	PSPEEDE	0.916
367	COL1A2	Collagen alpha-2(I) chain	VGPAGPRGPAG	0.917
368	LRRN1	Leucine-rich repeat neuronal protein 1	LNDLQQLNLSGNNLTA	0.920
369	SYNE2	Nesprin-2	HSQAVTLIQEKMT	0.920
370	FGA	Fibrinogen alpha chain	PVPDLVPGNF	0.922
371	ITGA2B	Integrin alpha-IIb	LPEPEQP	0.923
372	APOA2	Apolipoprotein A-II	VELGTQPA	0.925
373	SMARCA2	Probable global transcription activator SNF2L2	PGPILGPSPGP	0.931
374	FGA	Fibrinogen alpha chain	GDFLAEGGGVR	0.934
375	AHSG	Alpha-2-HS-glycoprotein	PGLIYRQPN	0.935
376	COL1A1	Collagen alpha-1(I) chain	HDGGRYYRA	0.936
377	FRAT2	GSK-3-binding protein FRAT2	PPAVPLLLP	0.938
378	COL4A3	Collagen alpha-3(IV) chain	HLGPAGPEG	0.940
379	DYNC1H1	Cytoplasmic dynein 1 heavy chain 1	NAERVLLTTQG	0.940
380	MGP	Matrix Gla protein	ESMESYELN	0.940
381	LYZ	Lysozyme C	IFQIN	0.941
382	VIM	Vimentin	YSLGSALRPSTSR	0.942
383	COMP	Cartilage oligomeric matrix protein	ETHQLRQA	0.942
384	VIM	Vimentin	LNLR	0.943
385	COL1A2	Collagen alpha-2(I) chain	GYDFGYDGDFY	0.944
386	VIM	Vimentin	VRQQYESVA	0.945
387	VIM	Vimentin	PGGVYATR	0.945
388	PLIN4	Perilipin-4	SGLVSSLQ	0.945
389	COL2A1	Collagen alpha-1(II) chain	LQYMRA	0.948
390	COL1A1	Collagen alpha-1(I) chain	GISVPGPMGPSG	0.950
400	VIM	Vimentin	GPGTASRPSSSRSYVTTSTRTY	0.950
401	CACNA1I	Voltage-dependent T-type calcium channel subunit alpha-1I	HLGPAGAAGP	0.950
402	COL1A1	Collagen alpha-1(I) chain	YDEK	0.950
403	RNASE1	Ribonuclease pancreatic	GSPYVPVHFDASVEDST	0.951
404	VIM	Vimentin	PGGVYATRSSAVRLR	0.952
405	PLA2G2A	Phospholipase A2, membrane associated	EAALSYG	0.952
406	COL11A2	Collagen alpha-2(XI) chain	EGPPGPEGPAGLIGPPGIQGNPGPV	0.957
407	AHSG	Alpha-2-HS-glycoprotein	LIYRQPN	0.957
408	MTR	Methionine synthase	AGLPNTFGDY	0.958
409	COL1A2	Collagen alpha-2(I) chain	YDGDFYRA	0.959
410	LIG3	DNA ligase 3	MITRVI	0.959
411	VIM	Vimentin	TRTYSLGSALRPSTSRSLY	0.959
412	AHSG	Alpha-2-HS-glycoprotein	IYRQPN	0.961
413	VIM	Vimentin	GPGTASRPSSSRSY	0.961
414	F2	Prothrombin	EYQTFFN	0.962
415	BGLAP	Osteocalcin	DCDELADHIGFQEAYRRFYGPV	0.963
416	COL1A1	Collagen alpha-1(I) chain	TGGISVPGPM	0.964
417	SPP1	Osteopontin	PVKQADSG	0.964
418	PLA2G2A	Phospholipase A2, membrane associated	EAALSYGFYG	0.965
419	PLA2G2A	Phospholipase A2, membrane associated	EAALSYGFY	0.966
420	VIM	Vimentin	SVDFSLADAIN	0.969
421	VIM	Vimentin	SVPGVRLLQDSVD	0.970
422	LYZ	Lysozyme C	DYGIFQIN	0.970
423	COL5A3	Collagen alpha-3(V) chain	TQDLGEK	0.972
424	HBA1	Hemoglobin subunit alpha	GAEALER	0.975
425	COL1A2	Collagen alpha-2(I) chain	SVGPVGPAGPIG	0.976
426	ANG	Angiogenin	PVHLDQSIFRRP	0.976
427	HBA1	Hemoglobin subunit alpha	AGEYGAEALE	0.977
428	AHSG	Alpha-2-HS-glycoprotein	PPDSHVLL	0.979
429	COL1A2	Collagen alpha-2(I) chain	YDGDFY	0.979

430	PLA2G2A	Phospholipase A2, membrane associated	YKFSNSG	0.980
431	PLIN1	Perilipin-1	EDEVATPAAPRPGFPAVPR	0.980
432	COL11A2	Collagen alpha-2(XI) chain	GDVGPMGPP	0.981
433	PLA2G2A	Phospholipase A2, membrane associated	VNFHR	0.981
434	BSN	Protein bassoon	SMADAAPPG	0.981
435	COL2A1	Collagen alpha-1(II) chain	AFAGLGPRE	0.983
436	COL1A2	Collagen alpha-2(I) chain	DFGYDGDFYR	0.983
437	F2	Prothrombin	EYQTFFNPRTFG	0.984
438	BGLAP	Osteocalcin	DHIGFQEAYRRFYGPV	0.984
439	SPP1	Osteopontin	VYGLR	0.984
440	LYZ	Lysozyme C	GYNTRATNYNAGDR	0.985
441	COL1A2	Collagen alpha-2(I) chain	GGGYDFGYDGDFYRA	0.985
442	AHSG	Alpha-2-HS-glycoprotein	PPSPPLGAPGLPPAG	0.985
443	BGLAP	Osteocalcin	LADHIGFQEAYRRFYGPV	0.985
444	FGB	Fibrinogen beta chain	VNDNEEGFFSA	0.986
445	VIM	Vimentin	SPGGVYATRSSAVR	0.986
446	VIM	Vimentin	TRTYSLGSALRPSTSRS	0.986
447	COL1A1	Collagen alpha-1(I) chain	ISVPGPMG	0.986
448	MSR1	Macrophage scavenger receptor types I and II	AHFGQGT	0.987
449	COL1A2	Collagen alpha-2(I) chain	YDFGYDGDFY	0.989
450	PLA2G2A	Phospholipase A2, membrane associated	ALSYG	0.990
451	CILP	Cartilage intermediate layer protein 1	LHGAVSLPGGAPA	0.991
452	PLA2G2A	Phospholipase A2, membrane associated	YGFY	0.992
453	MYO10	Unconventional myosin-X	LHAAIPPL	0.993
454	PLA2G2A	Phospholipase A2, membrane associated	GGRGSPKDA	0.993
455	ITGA2B	Integrin alpha-IIb	YFDGYW	0.994
456	REV3L	DNA polymerase zeta catalytic subunit	AGLESDGYRG	0.995
457	BGLAP	Osteocalcin	GAPVPYPD	0.996
458	BGLAP	Osteocalcin	FQEAYRRFYGPV	0.997
459	COL1A2	Collagen alpha-2(I) chain	YDFGYDG	0.997
460	COL2A1	Collagen alpha-1(II) chain	PDPLQYM	0.997
461	VIM	Vimentin	SPGGVY	0.998
462	VIM	Vimentin	GGPGTASRPS	0.998