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# Detection of bone and cartilage-related proteins in plasma of patients with a bone fracture using liquid chromatography-mass spectrometry

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Running title: Bone and cartilage-related proteins in human plasma

**Abstract**

Following bone fracture, a large number of growth factors, cytokines, and their cognate receptors involved in the repair process are active at the fracture site. To determine whether they appear in the patients' blood as candidate biomarkers for following the outcome of healing we analyzed in the plasma of 25 patients with an acute bone fracture following affinity plasma purification, SDS gel electrophoresis and liquid chromatography - tandem mass spectrometry (LC-MS/MS). Two hundred and thirteen nonredundant proteins were identified in the *in-gel* analysis of pooled plasma proteins. Gene ontology (GO) analysis indicated that a majority of detected proteins were of extracellular origin, whereas only a small number were of intracellular (cytosole and nucleus) origin. A significant proportion of detected proteins was involved in the cell growth and proliferation, transport and coagulation. Twelve proteins were potentially related to bone and cartilage metabolism, and several have not been previously identified in the plasma, including: TGF-β induced protein IG-$H_3$, cartilage acidic protein 1, procollagen C proteinase enhancer protein and TGF-β receptor III.

## Introduction

The blood is rich with a large amount of previously unstudied molecules that could reflect the ongoing physiologic state of various tissues. As blood flows through most of the tissues of the human body the origins of plasma proteins are various. In the complex mixture of a plasma proteome, albumin and other carrier proteins are present in a high abundance, as well as proteins that originate from circulating blood cells. Almost all cells in the body communicate directly or indirectly with blood and upon damage or cell death tissue-specific proteins are released into the bloodstream. Therefore, most of potential undiscovered biomarkers will be eventually found in the plasma fraction, where much less abundant proteins enter the blood from the surrounding tissue.

Bone undergoes continuous turnover and remodeling consisting of bone formation and bone resorption, two opposite and well-balanced processes. The various bone serum and urinary markers are usually classified according to the metabolic process indicating low and high, decreased or increased bone turnover [1].

Following fracture, a large number of growth factors, cytokines, and their cognate receptors involved in bone repair are highly expressed at the fracture site in the first hours following injury. It is presumed that some or all of these factors initiate active repair process acting on the cells of the bone marrow, periosteum, and external soft tissues adjacent to the fracture site. Skeletal tissues are the main source of such proteins, while some are released from associated inflammatory cells at the site of injury [2, 10].

In this study we analyzed proteins as candidate biomarkers expressed in the plasma of patients with an acute bone fracture. The plasma proteins of patients were characterized by SDS gel electrophoresis and affinity purification followed by tandem mass spectrometry LC-MS/MS.

Following identification of proteins those associated with bone and cartilage metabolism were singled out and analyzed. Some of characterized proteins have not yet been identified in the circulation and their presence or quantity could reflect the extent of injury and the success of the fracture repair.

## Materials and methods

Plasma collection

Human blood plasma samples were supplied by the Clinic of Traumatology in Zagreb. The approval for the collecting samples was obtained from the institutional Ethics Committee. Blood samples from 25 adult humans (21-60 years of age) of both genders with a single long bone fracture were drawn into syringes containing 3.8% sodium citrate to form an anticoagulant-to-blood ratio (v/v) 1:9. Plasma was obtained by centrifugation (15 min at 3000xg), and aliquots of each adult blood sample were pooled for the further analysis. Aliquot samples were stored at -80°C until analysis.

Affinity column purification

Pooled plasma of patients with a single-bone fracture (80ml) was diluted 2-fold with 10 mM sodium phosphate buffer (pH 7), and applied to a heparin Sepharose column (Amersham Pharmacia Biotech), previously equilibrated with 10 mM sodium phosphate buffer (pH 7). Bound proteins were eluted from the column with 10 mM sodium phosphate buffer (pH 7) containing 1 M and 2 M NaCl. Eluted fractions were precipitated with saturated ammonium sulfate (SAS) to a final concentration of 35%.

SDS gel electrophoresis and *in-gel* digestion

SDS-PAGE was run on a NuPAGE 10% Bis-Tris gel (Invitrogen, Carlsbad, USA) using MOPS SDS buffer system, and subsequently stained with Coomassie staining kit (NuPAGE, Invitrogen), as instructed by the manufacturer. After staining, each of the seven gel lanes was sliced in 12 pieces and the corresponding pieces were combined as indicated in Figure 1. The pieces were

then subjected to in-gel reduction, alkylation and trypsin digestion as described previously [4]. Gel pieces were washed two times with acetonitrile/25 mM $NH_4HCO_3$, reduced by incubation with 10 mM dithiothreitol (DTT) for 45 minutes at 56ºC and carboxyamidomethylated by incubation in 55 mM iodoacetamide for 45 minutes at room temperature. Trypsin (Promega) was added to dried gel pieces (150 ng per piece, diluted in 25 mM $NH_4HCO_3$) and incubated overnight at 37°C. Tryptic peptides were extracted with formic acid/acetonitrile/$H_2O$ (10:20:70); and 100% acetonitrile, dried and resuspended in trifluoroacetic acid/acetonitrile/$H_20$ (1:2:97) for MS analysis.

Mass spectrometry

Tryptic peptides were analyzed by a liquid chromatography-mass spectrometry (LC-MS). Agilent 1100 nanoflow HPLC system (Agilent Technologies) was coupled to a LTQ-Orbitrap mass spectrometer (Thermo Scientific) using a nano-electrospray LC-MS interface (Proxeon Biosystems). Peptides were loaded on a home-made 75 μm $C_{18}$ HPLC column in solvent "A" (0.5% acetic acid in Milli-Q water) and eluted with a 70-minute segmented linear gradient of 10-60% solvent "B" (80% acetonitrile, 0.5% acetic acid in Milli-Q water) at a flow rate of ca. 250 nL/min.

Mass spectrometer was operated in the positive ion mode. Each measurement cycle consisted of a full MS scan acquired in the orbitrap analyzer at a resolution of 60000, and MS/MS fragmentation of the five most-intense ions in the linear ion trap analyzer. To further improve mass accuracy, the lock-mass option was used as described previously [9]. This has resulted in a typical peptide average absolute mass accuracy of less than 1 ppm.

Peak lists were generated using in-house developed software (Raw2msm) [9], and searched against concatenated forward and reverse ("decoy") IPI human database (version 3.13) using

Mascot search engine (Matrix Science). Searches were done with trypsin specificity (2 missed cleavages allowed), carboxyamidomethylation as fixed modification, and oxidized methionine as variable modification. Precursor ion and fragment ion mass tolerances were 10 ppm and 0.5 Da, respectively.

Results of the database search were validated in the MSQuant software (http://msquant.sourceforge.net). Only peptides with a mass deviation lower than 5 ppm were accepted; two peptides were required for protein identification.

Gene ontology (GO) analysis was performed using ProteinCenter software package (Proxeon Biosystems).

## Results

Gene ontology analysis of characterized plasma proteins

Pooled plasma samples were subjected to heparin affinity chromatography to enrich for proteins specific for bone and cartilage, majority of which are known to have heparin binding domains. This has also partially removed highly abundant plasma proteins, such as albumin, immunoglobulins, transferin and haptoglobulin. Fractions of interest were collected, precipitated with ammonium sulphate and separated on 1D SDS-PAGE gel (Figure 1). Gel bands were excised, digested with trypsin and analyzed by LC-MS/MS. Peptide fragmentation spectra were searched against the human IPI protein database, and the results of the database search were validated using MSQuant software. Only peptides with a mass deviation lower than 5 ppm were accepted; two peptides were required for protein identification, which led to an overall false-positive rate of less than 1% at both the peptide and the protein level.

In total, two hundred and thirteen nonredundant proteins were identified in the in-gel analysis of pooled plasma proteins from patients with a single bone fracture and listed in Table 1.

Gene ontology (GO) analysis of plasma proteins showed that a majority (63.8%) of detected proteins were of extracellular origin, whereas only a small number (7.5%) were of intracellular (cytosole and nucleus) origin. Interestingly, we also detected a relatively high number (35.2%) of membrane related proteins (Figure 2 A).

According to the molecular function analysis, 37.6% of detected proteins had catalytic properties, 18.3% were classified as signal transducers, and 13.1% as transporters (Figure 2 B).

In terms of biological activity, a significant proportion of detected proteins were involved in the cell growth and proliferation (21.1%), transport (23.9%) and coagulation (13.1%) (Figure 2 C).

Identification of bone- and cartilage-related proteins

From the list of detected proteins we singled out 12 proteins which could be related to bone and cartilage metabolism (Table 2). Among them there were proteins not previously identified in the plasma, like cartilage acidic protein 1 (CRTAC-1) which was identified with 28 peptides and an average peptide Mascot score of 53. A molecule also related to the cartilage metabolism was the Splice isoform A of the proteoglycan-4 or lubricin, identified with 2 peptides and an average peptide Mascot score of 60.

Transforming growth factor beta receptor III was identified in the plasma for the first time with 4 specific peptides and an average Mascot score of 44, as well as the transforming growth factor beta induced protein IG-H3, with 20 peptides and an average peptide Mascot score of 57.

Among extracellular matrix proteins not previously detected in the plasma was the alpha 3 type VI collagen isoform 1 identified with 2 peptides and an average peptide Mascot score of 60.

Previously identified extracellular matrix proteins of interest for bone repair included: isoform long of collagen alpha-1 (XVIII) chain precursor or endostatin with 5 peptides and an average peptide Mascot score of 36, splice isoform 2 of collagen alpha 3 (VI) chain precursor with 10 identified peptides and an average peptide Mascot score of 62, extracellular matrix protein 1 precursor with 57 identified peptides and an average peptide Mascot score of 54, and type IV collagenease precursor or matrix metalloproteinase-2 (MMP2) with 3 identified peptides and an average peptide Mascot score of 74 (Table 2). MMP-2 degrades extra-cellular proteins and disrupts the subendothelial basement membrane, thus enabling the transmigration of inflammatory cells. Another metalloproteinase inhibitor 1 (TIMP-1) was identified with 5 peptides and an average peptide Mascot score of 49 (Table 2).

## Discussion

In this study we used state-of-the art proteomics techniques to characterize proteins in the plasma of patients with an acute bone fracture. Gene ontology showed a variety of different proteins, among which several have not been previously detected in the blood and could reflect the bone and cartilage stages of bone regeneration. Among them CRTAC-1, a glycosylated extracellular matrix molecule secreted by chondrocytes from the human articular cartilage. In the cell culture it was described as a candidate marker to distinguish the chondrocyte-like phenotype and activity from osteoblast-like and mesenchymal stem cells [15]. Thus its presence in the plasma of patients with an acute fracture could indicate the normal development and function of cartilaginous callus formation within the first week after the fracture and then its replacement by bone in the following weeks. In parallel CRTAC-1 could also indicate a concomitant joint cartilage injury immediately following an accident. In this way it may help distinguish between fractures with and without damaged joint cartilage, which would make CRTAC-1 an ideal marker for the various stages of the fracture repair. In the following study we need a precise time-related follow up of the plasma profile of CRTAC-1 in patients with a bone fracture with and without injured joint cartilage.

Splice isoform A of the proteoglycan-4 is a secreted, cytoprotective glycoprotein, a product of the gene proteoglycan 4 and a major component of the synovial fluid participating in the boundary lubrication of synovial fluids [11, 12, 13]. It prevents protein deposition onto cartilage from synovial fluid, controls the adhesion-dependent synovial growth, and inhibits the adhesion of synovial cells to the cartilage surface. It is highly expressed by synoviocytes and could serve as a marker of their activity following injury. It has been previously identified in the plasma [5].

The fracture healing process might be associated with a distinctive enzyme activity pattern at the fracture site, which may be reflected in their respective plasma/serum concentrations of various enzymes in their activity pattern. Thus, variations in the concentration of TIMP-1 and MMP-1 in the period following the fracture could have an important influence on the bone healing, as well as on other mechanisms leading to the development of a nonunion [6].

Discovery of circulating TßRIII was surprising since it is known that it has an essential role in the murine and chick development and that TßRIII knockout mice have an embryonic lethal phenotype. TßRIII acts as a TGF-ß co-receptor, concentrating ligand on the cell surface and enhancing ligand binding to the signaling TGF-ß receptor TßRII.[8] It is well known that transforming growth factor ß1 (TGF-ß1) and its receptor TßRII together with extracellular matrix proteins osteocalcin and collagen type I have an important role in the process of fracture healing. This result might add TßRIII to a list of novel biomarkers for following fracture repair. Recently, it was shown that TßRIII has also an important function as a suppressor of breast and prostate cancer progression [3, 16]. The possibility of following the cancer progression by detection of TßRIII in plasma should be further examined, especially knowing the role of TGFβ-1 and related family members in the progression of tumor growth and metastasis [14].

TGF-β IG-$H_3$ adhesion protein in plasma may play an important role in the cell-collagen interactions and binding to type I, II and IV collagens and may have an important role in the endochondral bone formation. It may also serve as a potential biomarker for the progression of successful bone healing. Additional studies will be needed to demonstrate the potential of these newly discovered plasma proteins as potential biomarkers for following the fracture healing and related disorders in humans.

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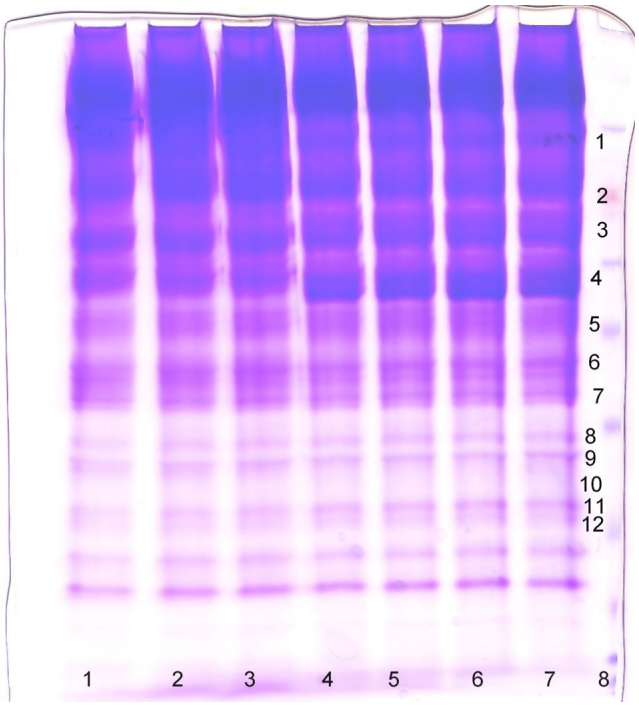
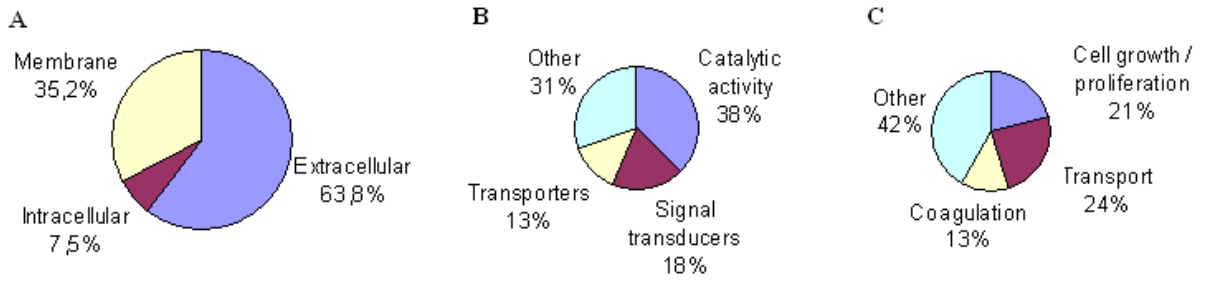
**Description of figures:**

Figure 1. Pooled plasma protein separation by one-dimensional SDS gel




Pooled plasma of patients with a single-bone fracture was applied to a heparin Sepharose column (Amersham Pharmacia Biotech). Bound proteins were eluted from the column with 10 mM sodium phosphate buffer (pH 7) containing 1 M NaCl ( lane 4-7) and 2 M NaCl (lane 1-3), lane 8 molecular mass marker . The numbers in the column indicate gel lanes sliced and prepared for MS analysis. Gel was stained with a Comassie Brilliant Blue.

Figure 2. Protein categorization using gene ontology (GO) component terms




Total nonredundant proteins identified from pooled plasma of patients with a single bone fracture were compared according to the following categories: (A) subcellular localization, (B) molecular function and (C) biological process.

Table 1. Proteins identified by a tandem mass spectrometry LC-MS/MS in pooled purified plasma from patients with fracture

| IPI Accession number | Protein name |
|---|---|
| IPI00000137.1 | N-acetylglucosamine-1-phosphotransferase subunit gamma precursor |
| IPI00000138.1 | Alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase |
| IPI00000816.1 | 14-3-3 protein epsilon |
| IPI00001753.1 | Myosin-4 |
| IPI00002147.4 | Chitinase-3-like protein 1 precursor |
| IPI00003176.1 | Serine protease HTRA1 precursor |
| IPI00003351.2 | Extracellular matrix protein 1 precursor |
| IPI00003590.2 | Isoform 1 of Sulfhydryl oxidase 1 precursor |
| IPI00004957.1 | Angiopoietin-related protein 3 precursor |
| IPI00006114.4 | Pigment epithelium-derived factor precursor |
| IPI00006154.1 | Isoform Long of Complement factor H-related protein 2 precursor |
| IPI00006510.1 | Tubulin beta-1 chain |
| IPI00006543.2 | Complement factor H-related 5 |
| IPI00006662.1 | Apolipoprotein D precursor |
| IPI00007118.1 | Plasminogen activator inhibitor 1 precursor |
| IPI00007199.4 | Protein Z-dependent protease inhibitor precursor |
| IPI00007221.1 | Plasma serine protease inhibitor precursor |
| IPI00007858.1 | Myosin-13 |
| IPI00008556.1 | Isoform 1 of Coagulation factor XI precursor |
| IPI00009865.1 | Keratin, type I cytoskeletal 10 |
| IPI00009920.2 | Complement component C6 precursor |
| IPI00010295.1 | Carboxypeptidase N catalytic chain precursor |
| IPI00010779.4 | Tropomyosin 4 |
| IPI00010896.2 | |
| IPI00011252.1 | Complement component C8 alpha chain precursor |
| IPI00011261.2 | Complement component C8 gamma chain precursor |
| IPI00011264.1 | Complement factor H-related protein 1 precursor |
| IPI00013004.1 | Isoform 1 of Pyridoxal kinase |
| IPI00016915.1 | Insulin-like growth factor-binding protein 7 precursor |
| IPI00017256.5 | Ras suppressor protein 1 |
| IPI00017530.1 | Ficolin-2 precursor |
| IPI00011264.1 | Complement factor H-related protein 1 precursor |
| IPI00018219.1 | Transforming growth factor-beta-induced protein ig-h3 precursor |
| IPI00018305.3 | Insulin-like growth factor-binding protein 3 precursor |

Table 1. Continued

| IPI Accession number | Protein name |
|---|---|
| IPI00019359.3 | Keratin, type I cytoskeletal 9 |
| IPI00019579.1 | Complement factor D precursor |
| IPI00019580.1 | Plasminogen precursor |
| IPI00019581.1 | Coagulation factor XII precursor |
| IPI00019591.1 | Isoform 1 |
| IPI00020091.1 | Alpha-1-acid glycoprotein 2 precursor |
| IPI00020996.3 | Insulin-like growth factor-binding protein complex acid labile chain precursor |
| IPI00021263.3 | |
| IPI00021304.1 | Keratin, type II cytoskeletal 2 epidermal |
| IPI00021364.1 | Properdin precursor |
| IPI00021439.1 | Actin, cytoplasmic 1 |
| IPI00021440.1 | |
| IPI00021727.1 | C4b-binding protein alpha chain precursor |
| IPI00021841.1 | |
| IPI00021842.1 | Apolipoprotein E precursor |
| IPI00021854.1 | Apolipoprotein A-II precursor |
| IPI00021885.1 | Isoform 1 of Fibrinogen alpha chain precursor |
| IPI00021891.5 | Isoform Gamma-B of Fibrinogen gamma chain precursor |
| IPI00022200.2 | alpha 3 type VI collagen isoform 1 precursor |
| IPI00022229.1 | Apolipoprotein B-100 precursor |
| IPI00022371.1 | Histidine-rich glycoprotein precursor |
| IPI00022391.1 | Serum amyloid P-component precursor |
| IPI00022392.1 | Complement C1q subcomponent subunit A precursor |
| IPI00022394.2 | Com |
| IPI00022395.1 | Complement component C9 precursor |
| IPI00022418.1 | Isoform 1 of Fibronectin precursor |
| IPI00022426.1 | AMBP protein precursor |
| IPI00022434.2 | ALB protein |
| IPI00022488.1 | Hemopexin precursor |
| IPI00022822.4 | Isoform Long of Collagen alpha-1(XVIII) chain precursor |
| IPI00022895.7 | Alpha-1B-glycoprotein precursor |
| IPI00022937.3 | Coagulation factor V |
| IPI00023006.1 | Actin, alpha cardiac muscle 1 |
| IPI00023728.1 | Gamma-glutamyl hydrolase precursor |

Table 1. Continued

| IPI Accession number | Protein name |
|---|---|
| IPI00024825.2 | Isoform A of Proteoglycan-4 precursor |
| IPI00025204.1 | CD5 antigen-like precursor |
| IPI00025276.1 | Isoform XB of Tenascin-X precursor |
| IPI00025862.1 | C4b-binding |
| IPI00026314.1 | Isoform 1 of Gelsolin precursor |
| IPI00026689.4 | Hypothetical protein DKFZp686L20222 |
| IPI00027507.1 | Complement factor H-related protein 3 precursor |
| IPI00027780.1 | 72 kDa type IV collagenase precursor |
| IPI00027827.2 | Extracellular superoxide dismutase [Cu-Zn] precursor |
| IPI00028091.2 | Actin-like protein 3 |
| IPI00028413.7 | Inter-alpha-trypsin inhibitor heavy chain H3 precursor |
| IPI00029061.2 | Selenoprotein P precursor |
| IPI00029193.1 | Hepatocyte growth factor activator precursor |
| IPI00029236.1 | Insulin-like growth factor-binding protein 5 precursor |
| IPI00029739.4 | Isoform 1 of Complement factor H precursor |
| IPI00029863.4 | Alpha-2-antiplasmin precursor |
| IPI00032179.2 | Antithrombin III variant |
| IPI00032220.3 | Angiotensinogen precursor |
| IPI00032258.4 | |
| IPI00032291.1 | Complement C5 precursor |
| IPI00032292.1 | Metalloproteinase inhibitor 1 precursor |
| IPI00032311.4 | Lipopolysaccharide-binding protein precursor |
| IPI00032328.1 | Isoform HMW of Kininogen-1 precursor |
| IPI00041065.3 | Hyaluronan-binding protein 2 precursor |
| IPI00043083.1 | Beta-parvin |
| IPI00060715.1 | BTB/POZ domain-containing protein KCTD12 |
| IPI00154742.5 | 25 kDa protein |
| IPI00163207.1 | Isoform 1 of N-acetylmuramoyl-L-alanine amidase precursor |
| IPI00164623.4 | 187 kDa protein |
| IPI00165438.2 | Muscle type neuropilin 1 |
| IPI00168728.1 | FLJ00385 protein (Fragment) |
| IPI00178083.2 | 29 kDa protein |
| IPI00183968.4 | tropomyosin 3 isoform 1 |
| IPI00186903.3 | Isoform 2 of Apolipoprotein-L1 precursor |

Table 1. Continued

| IPI Accession number | Protein name |
|---|---|
| IPI00216134.3 | tropomyosin 1 alpha chain isoform 7 |
| IPI00216699.1 | Isoform 2 of Unc-112-related protein 2 |
| IPI00216773.4 | ALB protein |
| IPI00218192.2 | Isoform 2 of Inter-alpha-trypsin inhibitor heavy chain H4 precursor |
| IPI00218732.2 | Serum paraoxonase/arylesterase 1 |
| IPI00219018.6 | Glycer |
| IPI00219465.4 | Transcobalamin-2 precursor |
| IPI00219682.5 | Erythrocyte band 7 integral membrane protein |
| IPI00219713.1 | Isoform Gamma-A of Fibrinogen gamma chain precursor |
| IPI00220327.2 | Keratin, type II cytoskeletal 1 |
| IPI00220350.1 | Isoform Beta-3B of Integrin beta-3 precursor |
| IPI00220642.6 | 14-3-3 protein gamma |
| IPI00220644.8 | pyruvate kinase 3 isoform 2 |
| IPI00220701.3 | Isoform 2 of Collagen alpha-3(VI) chain precursor |
| IPI00289831.4 | Isoform PTPS of Receptor-type tyrosine-protein phosphatase S precursor |
| IPI00291262.3 | Clusterin precursor |
| IPI00291866.5 | Plasma protease C1 inhibitor precursor |
| IPI00291867.3 | Complement factor I precursor |
| IPI00292218.3 | Hepatocyte growth factor-like protein precursor |
| IPI00292530.1 | Inter-alpha-trypsin inhibitor heavy chain H1 precursor |
| IPI00292950.4 | Heparin cofactor 2 precursor |
| IPI00293925.2 | Isoform 1 of Ficolin-3 precursor |
| IPI00294004.1 | Vitamin K-dependent protein S precursor |
| IPI00294193.4 | Isoform 1 of Inter-alpha-trypsin inhibitor heavy chain H4 precursor |
| IPI00294395.1 | Complement component C8 beta chain precursor |
| IPI00295976.5 | Isoform 1 of Integrin alpha-IIb precursor |
| IPI00296099.6 | Thrombospondin-1 precursor |
| IPI00296165.5 | Complement C1r subcomponent precursor |
| IPI00296176.2 | |
| IPI00296537.3 | Isoform C of Fibulin-1 precursor |
| IPI00296608.6 | Complement component C7 precursor |
| IPI00297284.1 | Insulin-like growth factor-binding protein 2 precursor |
| IPI00297550.7 | Coagulation factor XIII A chain precursor |

Table 1. Continued

| IPI Accession number | Protein name |
|---|---|
| IPI00297779.6 | T-complex protein 1 subunit beta |
| IPI00298497.3 | Fibrinogen beta chain precursor |
| IPI00298828.3 | Beta-2-glycoprotein 1 precursor |
| IPI00298860.5 | Growth-inhibiting protein 12 |
| IPI00298971.1 | Vitronectin precursor |
| IPI00298994.5 | 271 kDa protein |
| IPI00299145.8 | Keratin, type II cytoskeletal 6E |
| IPI00299547.4 | Neutrophil gelatinase-associate |
| IPI00299738.1 | Procollagen C-endopeptidase enhancer 1 precursor |
| IPI00302592.2 | filamin 1 |
| IPI00303476.1 | ATP synthase subunit beta, mitochondrial precursor |
| IPI00303963.1 | C |
| IPI00304273.2 | Apolipoprotein A-IV precursor |
| IPI00304865.3 | transforming growth factor, beta receptor III |
| IPI00305461.2 | Inter-alpha-trypsin inhibitor heavy chain H2 precursor |
| IPI00306311.8 | Pleckstrin |
| IPI00328609.3 | Kallistatin precursor |
| IPI00328703.1 | OAF homolog |
| IPI00329775.7 | Isoform 1 of Carboxypeptidase B2 precursor |
| IPI00333828.4 | Serpin A11 precursor |
| IPI00339228.1 | Isoform 8 of Fibronectin precursor |
| IPI00382436.1 | Ig lambda chain V-III region SH |
| IPI00382606.1 | Factor VII active site mutant immunoconjugate |
| IPI00383111.2 | 57 kDa protein |
| IPI00384280.5 | Prenylcysteine oxidase precursor |
| IPI00384444.4 | Keratin, type I cytoskeletal 14 |
| IPI00384938.1 | Hypothetical protein DKFZp686N02209 |
| IPI00385429.1 | collectin sub-family member 11 isoform b |
| IPI00387025.1 | Ig kappa chain V-I region DEE |
| IPI00387099.1 | Ig kappa chain V-I region Rei |
| IPI00387113.1 | Ig kappa chain V-III region B6 |
| IPI00387120.1 | Ig kappa chain V-IV region Len |
| IPI00399007.5 | Hypothetical protein DKFZp686I04196 (Fragment) |
| IPI00418153.1 | Hypothetical protein DKFZp686I15212 |

Table 1. Continued

| IPI Accession number | Protein name |
|---|---|
| IPI00418163.3 | complement com |
| IPI00418495.4 | Platelet |
| IPI00419424.3 | IGKV1-5 protein |
| IPI00426051.3 | Hypothetical protein DKFZp686C15213 |
| IPI00430808.1 | Hypothetical protein |
| IPI00430842.3 | IGHA1 protein |
| IPI00431645.1 | HP protein |
| IPI00448925.3 | IGHG1 protein |
| IPI00451624.1 | Isoform 1 of Cartilage acidic protein 1 precursor |
| IPI00465248.5 | enolase 1 |
| IPI00465378.1 | Apolipoprotein A-V precursor |
| IPI00465439.4 | |
| IPI00472073.1 | HLA class I histocompatibility antigen, B-59 alpha chain precursor |
| IPI00472610.2 | IGHM protein |
| IPI00477090.5 | IGHM protein |
| IPI00477597.1 | Isoform 1 of Haptoglobin-related protein precursor |
| IPI00477644.2 | 26 kDa protein |
| IPI00477992.1 | complement component 1, q subcomponent, B chain precursor |
| IPI00478003.1 | Alpha-2-macroglobulin precursor |
| IPI00478493.3 | HP protein |
| IPI00479116.1 | Carboxypeptidase N subunit 2 precursor |
| IPI00479708.5 | IGHM protein |
| IPI00549291.4 | IGHM protein |
| IPI00550991.3 | Isoform 1 of Alpha-1-antichymotrypsin precursor |
| IPI00553177.1 | Alpha-1-antitrypsi |
| IPI00641368.4 | Tsukushi precursor |
| IPI00641737.1 | Haptoglobin precursor |
| IPI00643034.2 | Isoform 1 of Phospholipid transfer protein precursor |
| IPI00643041.2 | GTP-binding nuclear protein Ran |
| IPI00643525.1 | Complement component 4A |
| IPI00646909.2 | Tubulin alpha-8 chain |
| IPI00654888.3 | Kallikrein B, plasma (Fletcher factor) 1 |
| IPI00719373.1 | IGLC1 protein |
| IPI00745872.2 | Isoform 1 of Serum albumin precursor |

Table 1. Continued

| IPI Accession number | Protein name |
|---|---|
| IPI00783024.1 | Myosin-reactive immunoglobulin heavy chain variable region (Fragment) |
| IPI00783987.1 | Complement C3 precursor (Fragment) |
| IPI00784822.1 | Hypothetical protein |
| IPI00785050.1 | Hypothetical protein |
| IPI00785200.1 | Hypothetical protein |
| IPI00787629.1 | similar to Apolipoprotein |
| IPI00790993.1 | 104 kDa protein |
| IPI00794487.1 | Immunoblobulin light chain (Fragment) |
| IPI00807531.1 | IGHG1 protein |

Table 2.

| Protein name | IPI accession number | Number of identified peptides | Average peptides Mascot score | Previously dentified in plasma | GO console: Molecular function | GO console: Cellular component | GO console: Biological Process |
|---|---|---|---|---|---|---|---|
| Extracellular matrix protein 1 precursor | IPI00003351.2 | 57 | 54 | + | -Signal transducer activity<br>-Structural molecule activity<br>-transporter activity | Extracellular | -cell communication<br>-metabolism<br>-regulation of biological process<br>-transport |
| Transforming growth factor beta induced protein IG-H3 precursor | IPI 00018219.1 | 20 | 57 | - | -protein binding | Extracellular | -cell proliferation<br>- regulation of biological process<br>-sensory perception |
| Splice isoform 1 of cartilage acidic protein 1 precursor | IPI00451624.1 | 28 | 53 | - | -metal ion binding | -proteasom<br>-Golgi complex | |
| Splice isoform 2 of collagen alpha 3 (VI) chain precursor | IPI00220701.3 | 10 | 62 | + | -enzyme regulator activity<br>-protein binding<br>- Structural molecule activity | Extracellular | -development<br>-transport |
| Type IV collagenease precursor | IPI00027780.1 | 3 | 74 | + | - catalytic activity<br>- enzyme regulator activity<br>- Metal ion binding - | Extracellular | -development<br>-metabolism |
| Alpha 3 type VI collagen isoform 1 precursor | IPI00022200.2 | 2 | 60 | - | -enzyme regulator activity<br>-protein binding<br>-Structural molecule activity | Extracellular | -development<br>-transport |
| Procollagen C proteinase enhancer protein precursor | IPI00299738.1 | 10 | 58 | - | - nucleic acid binding<br>-protein binding | Extracellular | -development<br>-metabolism |
| Transforming growth factor beta receptor III | IPI00304865.3 | 4 | 44 | - | -receptor activity<br>-signal transducer activity | -Golgi | -cell communication<br>-development |

Table 2. continued

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Isoform Long of Collagen alpha-1(XVIII) chain precursor | IPI00022822.4 | 5 | 36 | + | -Metal ion binding<br>-protein binding<br>-structural molecular activity | Extracellular | -cell death<br>-cell motility<br>-cell organization and biogenesis<br>-cell proliferation<br>-development<br>-regulation of biological process<br>-sensory per ception<br>-transport |
| Hyaluronan binding protein 2 precursor | IPI00041065.3 | 7 | 51 | + | - catalytic activity | Extracellular | -metabolism |
| Metalloproteinase inhibitor 1 precursor | IPI00032292.1 | 5 | 49 | + | -catalytic activity<br>-enzyme regulator activity<br>-Metal ion binding | Extracellular | -cell proliferation<br>-development<br>-regulation of biological process<br>-metabolism |
| Splice isoform A of proteoglycan-4 precursor | IPI00024825.2 | 2 | 60 | + | | Extracellular | Cell proliferation |

Individual peptide Mascot scores > 27 indicate identity or extensive homology ($p<0.05$).