Published in final edited form as:

Curr Proteomics. 2010 April 1; 7(1): 49–65.

DIFFERENTIAL SERUM LEVEL OF SPECIFIC HAPTOGLOBIN ISOFORMS IN SMALL CELL LUNG CANCER

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Abstract

Lung cancer is the leading cause of cancer death for both men and women in the United States, and similar trends are seen world wide. The lack of early diagnosis is one of the primary reasons for the high mortality rate. A number of biomarkers have been evaluated in lung cancer patients, however, their specificity and early stage diagnostic values are limited. Using traditional protein chemistry and proteomics tool we have demonstrated higher serum haptoglobin levels in small cell lung cancer (SCLC). Similar findings have been reported for other cancers including ovarian cancer and glioblastoma. Haptoglobin is an acute phase protein with at least six possible phenotypes. The six phenotypes, in combination with two post translational modifications, glycosylation and deamidation, lead to large numbers of possible haptoglobin isoforms. Recent studies indicate a possible correlation between specific haptoglobin glycosylation and particular disease conditions. In our current study, we have fractionated control and SCLC patient serum by 2-D gel electrophoresis to identify differentially expressed haptoglobin isoforms in SCLC serum samples.

Keywords

Serum; Haptoglobin; Isoforms; Small cell lung cancer

Introduction

Lung cancer is the most common cause of cancer death world wide, with over one million cases annually (WHO, economics of tobacco control). For 2009, it is predicted that 219,440 new cases of lung cancer will be diagnosed in the United States, 15% of which are small cell lung cancer (SCLC) and the rest non-small cell lung cancer (NSCLC) [1]. [1]. The overall survival for patients with SCLC is quite poor. Approximately 1/3 of patients initially present with limited disease (LD, within the chest cavity) their survival rate is only 20–25% over 5 years. A majority of SCLC patients present with extensive disease (ED, metastatic) and their chances of survival is less than 5% over 5 years. The methods of detecting early SCLC are poor and better strategies are urgently needed. Serum biomarkers for lung cancer have been studied in the hope of achieving early detection of the disease, improving diagnosis, predicting

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response or monitoring recurrence after treatment [2]. Nonetheless, their present clinical usefulness remains limited [3,4]. CEA, α -1-acid glycoprotein (AGP), neuron-specific enolase (NSE), chromogranin A (ChrA), bombesin-like gastrin-releasing peptide (GRP), and BB isoenzyme of creatine kinase (CK-BB) are some of the candidate serum biomarker for SCLC [5]. Many potentially useful candidates continue to be tested, especially serum cytokines. These include vascular endothelial growth factor (VEGF), stem cell factor (SCF) and hepatocyte growth factor/scatter factor (HGF/SF) [32]. However the clinical applications of all these serum biomarkers are limited.

Haptoglobin is an acute phase protein and has been reported to be indicative of various pathological conditions including diverse forms of cancer, cirrhosis of the liver, and hepatitis-C [6–8]. It binds to hemoglobin and is a marker for hemolysis [9]. Haptoglobin has also been shown to inhibit prostaglandin synthesis and angiogenesis and it constitutes about 0.4–2.6% of total serum protein [10,11]. Native haptoglobin is a heterotetramer composed of two α and two β subunits attached by disulfide bridges [12]. The human β subunit is a 38 kDa polypeptide linked to α isoforms by a disulfide bridge. There is only one type of β subunit, while the α chain is represented by two isoforms α -1 and α -2. The amino acid sequences in α isoforms are similar, with the α -1 isoform having 83 amino acids (9-kDa polypeptide). The α -2 isoform is a duplicate of the α -1 chain, with a repeat insert of amino acid residues 12 to 70, thus the α -2 chain has 142 amino acids (20 kDa polypeptide). One of the distinct features of distinct feature of β -haptoglobin is its high level of glycosylation. The β -haptoglobin chain has 243 amino acids, with a molecular mass of more than 40 kDa. It is estimated that the β chain contains approximately 30% carbohydrate moiety [13,14].

We have reported a higher circulatory level of α haptoglobin in SCLC patients. Our studies also indicated a possible correlation between the disease state and the level of α -haptoglobin in serum [15]. The present study is an extension of our earlier work In this study we have analyzed serum and haptoglobin enriched serum fractions to determine the differential expression of specific haptoglobin isoforms in SCLC patient serum. The samples were analyzed by two dimensional gel electrophoresis (2D-GE) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Once fractionated, the proteins were characterized either by immunoblotting or silver stain in combination with mass spectrometry. The present study confirms our earlier findings that higher serum levels of α and β haptoglobin isoforms were observed in SCLC patient samples. Also, we have demonstrated a β chain variant (5 kDa smaller than the full length) differentially expressed in SCLC patient serum.

Materials and methods

Serum Sample Collection

Serum from control or SCLC patients were collected as described earlier (4), with institutional review board (IRB) approved informed consent.

Serum haptoglobin enrichment by albumin and immunoglobulin depletion

To remove albumin, the method developed by Colantonio et. al was followed with modifications [16]. Briefly, serum samples of 100 to 500 μ l were incubated with 0.1 M NaCl final concentration for 1 hour at 4°C. Cold ethanol was added to the sample to reach a final concentration of 42% and was incubated at 4°C for 1 hour. The mixture was then centrifuged at 16,000 g for 45 minutes; both the pellet and supernatant were retained in separate micro tubes. The pellet was washed in 42% ethanol and centrifuged at 16,000 g for 45 minutes. The pellet was retained, and the second supernatant was discarded. The pH of the first supernatant collected after ethanol precipitation was reduced to 5.7 using cold 0.8 M sodium acetate (pH 4.0), and incubated at 4°C for 1 hour. This mixture was then centrifuged at 16,000 g for 45

minutes. Once again, the pellet and supernatant were retained. The two pellets were re-suspended in a triton-X lysis buffer, and the pooled supernatant with albumin was used for analytical purposes.

The re-suspended pellets were then further processed in order to remove immunoglobulins using iron nanoparticles. Briefly, protein A coated iron nanoparticles (Miltényi, Inc.) were added to the albumin-free serum sample protein mixture and incubated at RT for one hour. A microcolumn was equilibrated with 200 μ l of lysis buffer. The sample with protein A nanoparticles was then loaded into the column and allowed to elute, while the nanoparticles with attached immunoglobulins were retained within the magnetic column. The immunoglobulin-free protein sample was then subject to the TCA/Acetone precipitation for 2D-GE.

2D Gel Electrophoresis

Tri-chloro acetic acid (TCA) /Acetone Precipitation—TCA/Acetone precipitation was performed to optimize serum protein recovery. 100 μ l of serum was mixed with 800 μ l of ice-cold acetone and 100 μ l of 100% TCA. Following incubation at -20°C for one hour and centrifugation steps, the precipitated protein pellet was dissolved in 250 μ l of two-dimensional gel rehydration buffer (8 M urea, 2 % (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate) (CHAPS), 50 mM dithiothreitol (DTT) (Sigma Aldrich), and 0.2% Bio-lyte ampholyte (Bio-Rad).

Serum Protein Quantification—In order to achieve equal loading for 2D-GE the protein concentration of the samples solubilized in rehydration buffer were determined using a Bio-Rad RC/DC kit (manufacturer's protocol was followed).

Rehydration of IPG Strip for 2D Electrophoresis—A known amount of protein was diluted to make 125 μ l with rehydration buffer (8M urea, 2% CHAPS, 50mM DTT, and 0.2% Bio-lyte ampholyte). The IPG strips (Bio-Rad); pI 4 -7 were rehydrated with 125 μ l sample for 11–16 hours.

First Dimensional Analysis Using Isoelectric Focusing—Isoelectric focusing (IEF) was done using a Protean IEF Cell (Bio-Rad). The IEF run was performed using the following protocol: 100 V for 3 hours, 300 V for 2 hours, 600 V for 1 hour, 1000 V for 1 hour, 2000 V for 1 hour, and 3000 V for 8 hours.

Second Dimensional Analysis by SDS-PAGE—Following the IEF, the IPG strips were equilibrated in two different buffers. The buffer solutions were comprised of 6M urea, 0.375 M Tris-HCl, pH 8.8, 2% SDS, 20% glycerol and 2% DTT in the first equilibration buffer or 2.5% iodoacetamide instead of DTT in the second equilibration buffer. The IPG strips were equilibrated for 20 minutes in each equilibration buffer. The proteins were then further fractionated on SDS-PAGE gel and were analyzed either by silver staining or immunoblot analysis.

Immunoblotting with Anti-haptoglobin antibody

To perform an immunoblot analysis for haptoglobin, the serum samples were analyzed by 12.5% SDS-PAGE and immunoblotting was performed with anti haptoglobin antibody (Sigma). Results were visualized using chemiluminescent reagents (Perkin Elmer) and autoradiography.

Mass Spectrometry

Proteins from the silver stained gel were excised and processed for in gel digestion as described [17,18]. Briefly, gels were cut into small, uniform pieces; the gel pieces were dehydrated by acetonitrile and then rehydrated by 100 mM ammonium bicarbonate. Samples were treated with 10 mM DTT to reduce disulphide bonds followed by 50 mM iodoacetamide to block disulphides from reforming. Gel pieces were then washed with ammonium bicarbonate and dried by acetonitrile and these last two steps were repeated twice. After completely dehydrating with acetonitrile, gel pieces were suspended in 12.5 ng/μl trypsin in 50 mM ammonium bicarbonate. In-gel digestion was carried at 37°C for 10–12 hours and the peptides were extracted from the gel in 50% acetonitrile and 5% formic acid. The extract was concentrated under reduced pressure and finally desalted by C-18 containing Zip-Tip (Millipore, MA). Trypsin digested peptides were analyzed by ES-MS-MS and MALDI-TOF-TOF-MS (ABI-4800). MALDI-TOF-TOF-MS analyses were performed using the following parameters: source 1 voltage at 80 kV, source 2 voltage at 15 kV, source 1 focus at 4.3 kV, source 1 lens at 3.7 kV, Y1 deflector at 0.08 kV, grid source 1 voltage ratio of 0.91, mirror 2 to mirror 1 voltage ratio of 1.7, and the mirror 2 to source 2 voltage ratio of 1.23. MS spectra were recorded in a mass range of 800–4000 Daltons. Data was collected and analyzed using the Explorer software package (ABI).

Mass Spectrometry Data Analysis

Mass spectrometry data was analyzed and interpreted using the MASCOT (ver. 2.0.04) and Protein Prospector (ver. 4.27.2 basic and ver. 5.0, UCSF) database search programs. The database search parameters were as follows: for the NCBI database; SwissProt, tryptic digest, 2 missed cleavages, and a mass tolerance of 20–50 ppm. MS/MS data was analyzed by MASCOT.

Results

Differentially expressed haptoglobin isoforms in SCLC patient serum

To determine the differential expression of specific isoforms, we fractionated serum samples by 2D-GE and estimated quantitatively various haptoglobin isoforms by immunoblot analysis with anti haptoglobin antibody. Serum from control (pooled serum from normal individuals) and SCLC patients (eight representing stage III and Stage IV) were acetone precipitated and equal amount of proteins were analyzed. The immunoblot analysis with anti haptoglobin antibody demonstrated the presence of β , α -1 and α -2 chains in control and SCLC patients as well. Both α and β isoforms were elevated in SCLC patient samples. The streak of haptoglobin gave a very strong signal and resulted in a thick haptoglobin bands at 45 kDa (β chain) 19 kDa (α -2 chain) and 9kDa (α -1 chain) (fig. 1A and B). A comparative analysis of control and SCLC serum samples also showed a set of three spots, 5 kDa smaller than haptoglobin β chain and pIs ranging from 5 to 6 (Fig 1B, marked a,b,c,d). These haptoglobin minor isoforms were not visualized in control serum samples. The quantitative estimation of these spots indicate variation between patients and there was distinct difference between stage III and IV patient sera (table 1).

Haptoglobin enrichment by ethanol precipitation

To further characterize low abundance minor isoforms, we first partially enriched haptoglobin by removing albumin and immunoglobulin. A combination of ethanol/sodium acetate precipitation for albumin, and protein A based removal of immunoglobulin was optimized. The haptoglobin enriched samples were analyzed by SDS-PAGE and coomassie stain. The results clearly indicate significant removal of IgG and albumin (fig. 2A). Most of the albumin was removed and collected as a separate fraction. To determine the loss of haptoglobin during

fractionation, we analyzed various fractions by immunoblot analysis with an anti-haptoglobin antibody. Immunoblot analysis of post-enrichment fractions clearly demonstrated the complete absence of haptoglobin in the albumin fraction. All three major isoforms (45 kDa β , 19 kDa α -2 and 9 kDa α -1 chain of haptoglobin) were visualized (fig. 2B).

2D-gel electrophoresis analysis of haptoglobin enriched serum

To fractionate and identify different haptoglobin isoforms, haptoglobin enriched serum samples were analyzed by two-dimensional gel electrophoresis, followed by SDS-PAGE. To visualize the proteins, the SDS-PAGE was subjected to silver staining. 2D-GE of haptoglobin enriched control serum samples showed a streaking pattern of three major proteins, albumin (64 kDa), the immunoglobulin heavy chain, and both the α and β chains of haptoglobin (fig. 3A). Several silver stain protein spots of the gel were excised and analyzed by MS for protein identification.

Characterization of different haptoglobin isoforms

Haptoglobin streak spots were visualized at 45 kDa, 19 kDa and 9 kDa representing β , α -2 and α -1 isoforms. The characterization of protein at 45 kDa resulted in the identification of haptoglobin isoforms and two spots with similar mass and pI were identified as apolipoprotein (fig. 3B and table 1). The pI of the β isoform ranged from 5.5 to 6.5. Four haptoglobin isoforms of α -2 with masses of approximately 19 kDa were also identified. The pI range of the α -2 isoform varied from 4.5 to 6.5 (fig. 3B). Two spots of α 1 isoform with a molecular mass of 9 kDa were also identified by MS analysis in control and patient serum samples (fig. 3A and B). The protein identification was based on ES-MS/MS or MALDI-TOF-TOF-MS analysis. At least four representative peptides and at least two representative peptide MS/MS were considered minimal to confirm the identification of the proteins. The major haptoglobin isoforms were represented by several peptides and MS/MS data of most of the peptides showed significant y and b ion fragments (fig. 5).

Haptoglobin isoforms differentially expressed in SCLC patient serum

In a series of experiments, we fractionated haptoglobin enriched serum samples by 2D-GE to identify and characterize differentially expressed haptoglobin isoforms. A comparative analysis of control and SCLC serum samples by 2D-GE and silver stain showed similarity however two distinct differences were striking. Higher level of haptoglobin and the presence of 40 kDa minor haptoglobin isoforms were notable. Only SCLC patient sera showed an elevated level of haptoglobin, while also containing two 40 kDa isoforms of the β chain (fig. 4A and B). Differentially expressed 40 kDa isoforms were negatively stained with silver. One of the spots was identified as haptoglobin by MS analysis however the identification of the second protein remained inconclusive. Our studies also indicate that these proteins cross reacts with anti-haptoglobin antibody (fig. 1B spot c).

Discussion

To determine the differential expression of haptoglobin isoforms we analyzed control and SCLC patient serum by 2D-GE. The fractionated proteins were either characterized by silver stain and MS analysis or by immunoblot analysis with anti-haptoglobin antibody. Comparison of 2D-GE and immunoblot analysis recorded two important observations, that both the β and α haptoglobin isoforms were elevated in patient sera, and also most strikingly that there were differentially expressed β chain minor isoforms. Haptoglobin α chain heterogeneity was similar in control and SCLC patient sera. Using 2D-GE and MS-based protein characterization methods, it has been demonstrated that the mobility shift in α -1 and α -2 isoforms are due to the changes in amino acids leading to different charge states [20,21].

Acute phase proteins, such as haptoglobin has been shown to be significantly higher in the sera of patients with inflammatory diseases and cancer [22–24]. Haptoglobin is synthesized in the liver, however higher levels of haptoglobin has been observed in tumor tissues [25]. This may be due to the epithelial-mesenchymal transition phenomenon, and the fact that tumor tissue demonstrates similarities with inflammatory cells, particularly activated fibroblasts [26,27]. Several studies have reported a higher level of serum haptoglobin in a variety of cancers including: ovarian, breast, small cell lung, pancreatic, liver and prostate cancers [6–8,15,23–25,28]. The convergent themes of these studies are a higher circulatory level of serum haptoglobin and an altered glycosylation status of the β chain. However, there is no consensus on the specificity of differential expression of haptoglobin isoforms or specific glycans.

Higher levels of haptoglobin may be attributed to higher protein levels and/or increased glycosylation of the β isoform in particular. Saldova et. al have determined the correlation between cancer progression and isoform glycosylation. They have concluded that changes in glycoforms in haptoglobin β chain, α 1-acid glycoprotein and α 1-antichymotrypsin leads to changes in the total serum glycome of patients with advanced ovarian cancer. These changes include increases in levels of core fucosylated, agalactosyl biantennary glycans (FA2) and sialyl Lewis x (SLe x) [29]. Our observation of higher levels of the β isoform of haptoglobin further extends these findings.

The other significant difference between control and SCLC patient sera was the differential expression of minor isoforms of the β chain. There were two different sets of spots, several minor isoforms 1–2 kDa below the 45 kDa β isoform (will be referred to as the 44 kDa isoform) with basic pIs between 5.5 and 7.0. The other set with 3–5 spots approximately 5 kDa smaller than the 45 kDa β isoform was observed only in patient samples (referred to as the 40 kDa isoforms) (fig. 1). The pIs of these isoforms were acidic, between 4.5 and 6.0. The 44 kDa minor β haptoglobin isoforms appear in doublet and have also been observed in alcoholic and cirrhotic patients [30]. Gravel et. al has attributed these changes to altered glycosylation. They have demonstrated the loss of terminal sialic acid, the galactose residue and the N-acetylglucosamine residue which are located at the penultimate and antepenultimate position of the glycan chains, respectively. However, He et al concluded that sialic acid content of major isoforms determine their separation by isoelectric focusing, but the analysis of doublets of minor isoforms indicated similar glycan structure, thus the heterogeneity in minor isoforms was due to modifications distinct from N-glycan structure [11]. In SCLC patients serum, we have also observed several 43–44 kDa minor isoforms with altered mobility towards basic pIs. Micro-characterization of these isoforms will be important to determine their correlation to SCLC disease progression.

The second haptoglobin minor isoforms were 4–5 kDa smaller than the 45 kDa β chain and maintain acidic pIs (4.5 to 5.5). This haptoglobin isoform has not yet been reported in any other cancer or inflammatory disease. The relative abundance of these isoforms are very low and to further characterize these isoforms we enriched control and SCLC patients serum samples by removing albumin and immunoglobulins. Albumin was removed by ethanol precipitation as described by Colantonio et. al [16]. In their procedure, removal of highly abundant proteins such as albumin is coupled to powerful protein separation methods in order to increase sample loads, thus facilitating detection and identification of low abundance proteins. The haptoglobin enriched serum fractions from control and SCLC patients were analyzed by 2D-GE and silver staining. Two 40 kDa protein spots were visible only in SCLC patient sera. These spots were negatively stained indicating the acidic nature of the proteins [31]. One of the spots was identified as haptoglobin, while the other was identified as apolipoprotein. The other two minor haptoglobins identified by immunoblotting were not observed in silver stain gels, possibly due to the lack of comparative sensitivity of silver staining.

Hakomori and colleagues have characterized serum haptoglobin in their effort to determine the correlation between glycosylation status and prostate cancer progression [8]. They purified haptoglobin from control, benign and prostate cancer patients and characterized the glycans by mass spectrometry. They concluded that levels of haptoglobin are enhanced in sera of prostate cancer patients, and the N-glycans attached to a defined region of its β chain are characterized by enhanced branching as well as antenna fucosylation [8]. They further characterized their putative glycan structure, as well as determining their relative abundance. Most of the glycans except three were increased in patient sera. They have demonstrated the reduction in the level of these three glycans in patient sera. The mass of these glycans vary from 5 to 6 kDa. We are tempted to draw a parallel conclusion and characterize the 40 kDa minor haptoglobin isoform in our studies as reduction in certain glycans in SCLC patient serum as well. However, deletion of these glycans will significantly change the pI to basic, thus the loss of a glycan structure does not explain the differential expression of these minor β haptoglobin isoforms. These isoforms are recognized by anti-haptoglobin antibody and by mass spectrometry. The anti-haptoglobin antibodies are highly specific as they have very little or no cross reactivity with the most abundant proteins, i.e. albumin. Also, we have characterized at least one minor isoform by MS analysis. Taken together, these differentially expressed proteins represent haptoglobin isoforms. However, we have not yet fully characterized these isoforms. Currently we are purifying haptoglobin from control and SCLC patient sera to further characterize the 40 kDa minor haptoglobin isoforms.

Acknowledgments

We are grateful to Paul Morrison for providing us the resources at Blais Proteomics Center at the Dana-Farber Cancer Institute. This work was supported by a pilot grant from the Department of Medicine to Ajit Bharti and . 5R01CA100750-06 from National Cancer Institute to Ravi Salgia.

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A **B**

Figure 1. 2D-Gel electrophoresis and immunoblot analysis

A total of 500 μ g of serum protein from control (A) and SCLC patient (B) were analyzed by 2D-gel electrophoresis and immunoblot with anti-haptoglobin antibody. Serum proteins were first separated on pI 4–7 IPG strips and then on 12% SDS-PAGE. β , α -1 and α -2 haptoglobin chains were observed at 47, 9 and 19 kDa, respectively (indicated by arrows) in control or SCLC patient serum samples. In patient samples, two series of haptoglobin proteins were also recorded, one near the 47 kDa protein and the other 5 kDa lower, approximately at 42 kDa.

Figure 2. Haptoglobin enrichment by removal of albumin and immunoglobulins

Serum samples from control and SCLC patients were ethanol precipitated and immunoglobulin was removed by protein A affinity purification. Haptoglobin-enriched fractions were analyzed by SDS-PAGE and coomassie staining (A) and immunoblot analysis with anti-haptoglobin antibody (B).

Figure 3. Characterization of haptoglobin isoforms by 2D-SDS-PAGE and MS

Haptoglobin-enriched serum from control (A) and SCLC patient (B) were analyzed by 2D-GE and SDS-PAGE. The proteins were detected by silver staining. Protein spots (circled and numbered) from both control and SCLC were processed by in-gel digestion. Trypsin digested proteins were analyzed by MALD-TOF-TOF-MS. Several proteins, including ten haptoglobin isoforms were characterized (see Table 2).

A **B**

Figure 4. Characterization of differentially present/expressed haptoglobin isoforms in SCLC patient samples

Control (A) and SCLC patient serum samples (B) were enriched for haptoglobin. Haptoglobin enriched samples were analyzed by 2D-GE-SDS-PAGE and silver staining. Two sets of proteins, one close to the β chain and the other between the α -1 and α -2 chains were differentially present in SCLS patient serum samples only (shown in insets).

Peptide Sequence: TEGDGVYTLNNEK

B

Figure 5. Representative MS data from a spot identified as haptoglobin

Trypsin digest of the 40 kDa negatively stained spot (from figure 4b, lower inset) was analyzed by MS. MS spectrum representing several haptoglobin peptides (marked by arrow) was identified as of haptoglobin by mass fingerprinting (fig A). One specific haptoglobin peptide with an m/z of 1439 was further analyzed to generate MS/MS data. The sequence analysis resulted in a significant y and b series representing haptoglobin peptide (fig. B).

Table 1

Quantitative representation of different 40 kDa haptoglobin isoforms in SCLC patient serum.

Patient ID	Disease Stage	Size at Spot A	Size at Spot B	Size at Spot C	Size at Spot D
Control	n/a	–	–	–	–
120498	IV	+	++	++	++
1271205	IV	+	+++	+++	+++
1360287	IV	+	+	++	++
1382081	IIIB	+	+	+++	+++
1394305	IIIB	++	+++	+++	+++

Table 2

Spot Number	Protein Identification	Accession Number	Molecular Weight (kDa)	pI	Function
1	Haptoglobin-Related Protein Precursor	P00739	39.0	6.4	Acute-Phase Protein
2	Haptoglobin Precursor	P00738	45.2	6.1	Acute-Phase Protein
3	Haptoglobin Precursor	P00738	45.2	6.1	Acute-Phase Protein
4	Haptoglobin-Related Protein Precursor	P00739	39.0	6.4	Acute-Phase Protein
5	Apolipoprotein A-I Precursor	P02647	30.7	5.6	Primary Protein Constituent of HDL
6	Haptoglobin Precursor	P00738	45.2	6.1	Acute-Phase Protein
7	Apolipoprotein L-6	Q9BWW8	38.4	8.6	Acute-Phase Protein
8	Haptoglobin Precursor	P00738	45.2	6.1	Acute-Phase Protein
9	Haptoglobin Precursor	P00738	45.2	6.1	Acute-Phase Protein
10	Haptoglobin, Isoform CRA_a	I119579598	46.7	6.3	Acute-Phase Protein
11	Haptoglobin, Isoform CRA_a	I119579598	46.7	6.3	Acute-Phase Protein
12	Haptoglobin-Related Protein Precursor	P00739	39.0	6.4	Acute-Phase Protein