Advance Publication

Journal of Toxicologic Pathology

Received : August 26, 2018

Accepted : March 19, 2019

J-STAGE Advance Published Date : July 5, 2019

Japanese Society of Toxicologic Pathology
Proteomics Approach to Investigate Dynamic Protein Profile Involved in High Fat Diet–induced Fatty Liver Disease in Rats

Baohua Huang¹#, Yanling Yao¹#, Yaping Li¹, Hua Yang¹, Huchen Liu¹, Heng Liu², Dongming Li², Wei Shu²¶, and Ming Chen¹¶

¹State Key Laboratory for Chemistry and Molecular Engineering of Medical Resources, Guangxi Normal University, Guilin, China, 541004
²Department of Cell Biology and Genetics, Guangxi Medical University, Nanning 530021, P. R. China

Running Title: Dynamic protein profile in fatty liver

# Co-first authors of this article

¶Correspondence to:

Ming Chen, Ph.D., State Key Laboratory for Chemistry and Molecular Engineering of Medical Resources, Guangxi Normal University, Guilin, China, 541004

Email: chenmingprotein@163.com

Wei Shu, Ph.D., Department of Cell Biology and Genetics, Guangxi Medical University, Nanning, China, 530021

Email: Shuwei7866@126.com
Abstract
Nonalcoholic fatty liver disease (NAFLD) is a disorder of the liver found worldwide. The molecular mechanisms underlying NAFLD initiation and progression, however, remain poorly understood. In this study, fluorescence difference gel electrophoresis (DIGE) combined with mass spectrometry was performed to profile the intracellular processes in the rat liver at the proteome level when rats were fed a high-fat diet for 8 weeks. Dynamic changes of 27 protein spots were observed. Among them, upregulation of 14 spots and downregulation of 13 spots were observed during the eight weeks of the high fat diet-induction period. These spots were analyzed by matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometry (MALDI-TOF/TOF), and ultimately 24 proteins were identified with more than 95% confidence. Gene ontology (GO) annotation indicated that these proteins were implicated in the metabolism of carbohydrates, lipids, and amino acids. Four proteins were validated by western blot. Further functional studies on these dynamically changing proteins may lead to a better understanding of the mechanisms of high fat diet-induced fatty liver disease.

Key Words: DIGE; High fat diet; Nonalcoholic fatty liver disease; Proteomic
Introduction

The liver is one of the most important organs in humans and animals and plays many key roles in life processes\(^1,2\). As a major site of metabolism and detoxification, the liver is the system of choice in studies involving toxicoproteomics and metabolic disorders due to various pathological processes. Liver diseases such as hepatitis, hepatocirrhosis, and liver cancer are among the most common causes of death around the world\(^3,4\). It has been confirmed that the development of fatty liver is the main cause of many chronic liver diseases\(^5\).

Recently, nonalcoholic fatty liver disease (NAFLD), which is considered to be the most common liver disease, has been the focus of study\(^6-8\). It is well known that many factors, such as industrial toxins and hepatic viruses, lead to liver damage; what is worse, NAFLD may potentiate these processes\(^9,10\). It has been estimated that approximately 20\%–30\% of adults in the United States and other western countries have excess fat accumulation in their liver. In some developing countries, fatty liver disease also affects a large proportion of the country’s population\(^11,12\). However, the pathogenesis of NAFLD remains largely unknown.

In this study, we wanted to resolve the proteins that are involved in the pathogenesis of NAFLD in the HFD-induced rat liver. To this end, the fluorescent two-dimensional difference gel electrophoresis (2D-DIGE) technique, which was originally introduced to detect differences between two or more biologic samples\(^13,14\), was performed to monitor protein dynamic changes in the rat liver. Dynamic changes of 27 protein spots were found, and 24 proteins were identified with more than 95\% confidence. Further functional study of these dynamically changing proteins may lead to better understanding of the mechanisms of high fat diet-induced fatty liver disease.

Materials and methods

Animal model and experimental protocol

Adult male Sprague Dawley rats, 10 weeks old, weighing 170–200 g were purchased from the Center of Experimental Animals of Sun Yat-sen University (Guangzhou, China). The protocol was
approved by the Committee on Experimental Animal Management of Guangxi Normal University. Animals were housed in a pathogen-free environment at room temperature and maintained on rat chow and water for 10 days. After that, the rats were divided randomly into a normal diet group and four groups with high-fat diet (HFD) feeding for 2, 4, 6, and 8 weeks. The basic composition of the standard chow was 4% beef tallow, 15% alpha-corn starch, 14% casein, 1% vitamin mixture, 3.5% mineral mixture, 0.25% choline hydrogen tartrate, 0.18% L-cystine, and 0.0008% t-butylhydroquinone. High-fat diets had the following composition: 40% beef tallow, 15% alpha-corn starch, 14% casein, 10.5% beta-corn starch, 1% vitamin mixture, 10% sugar, 5% cellulose, 3.5% mineral mixture, 0.18% L-cystine, 0.25% choline hydrogen tartrate, and 0.0008% t-butylhydroquinone (W/W per 100 g diet). The body weight of each rat was recorded every week. Rats were fasted 24 hours before sacrifice (animals were euthanized by inhalation of CO\textsubscript{2}) after the last day of high-fat diet feeding. Livers were rapidly excised and washed with ice-cold PBS buffer three times. After that, the livers were cut into pieces of about 1–2 mm\textsuperscript{3} and then weighed and immediately frozen in liquid nitrogen. Blood samples were collected from rat hearts into evacuated tubes containing EDTA as an anticoagulant. Plasma was separated within 30 min at 4°C and stored at -80°C.

**Biochemical analysis**

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), and total cholesterol (TC) concentrations were determined using biochemical kits and methods as described by Zhang et al.\textsuperscript{16}. Triglycerides were assayed using a triglyceride assay kit (Triglyceride GPO-POD assay kit, Shanghai Zhen Biotechnology Co., Shanghai, China), and cholesterol was assayed with a rat cholesterol assay kit (Catalog # 79960, Crystal Chem USA, Elk Grove Village, IL, USA). A rat Alanine Aminotransferase (ALT/GPT) ELISA Kit was purchased from Qayee Bio-Technology Co., Ltd. (Catalog #QY-D0179, Shanghai, China). A rat Aspartate Aminotransferase (AST) ELISA kit was purchased from Cusabio Technology LLC (Code
Leptin was detected with a Rat ELISA Kit (Catalog #KRC2281, Thermo life, Shanghai, China). Glucose was detected with a Glucose Assay kit (Catalog #KA4088, Abnova).

**Histological analysis**

Liver specimens were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (H&E). Light microscopic examinations were performed.

**2D-DIGE and imaging**

Protein samples were pooled by treatment group for 2D-DIGE. Then, the pooled samples were dissolved in lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 30 mM Tris-HCL, pH 8.5) to produce stock solutions with final concentrations of approximately 5 mg/mL. Cyanine dyes were reconstituted in 99.8% anhydrous dimethyl formamide (DMF) and added to labeling reactions at a ratio of 400 pmol Cy dye to 50 μg protein in different groups following the cross-label rule, according to the manufacturer’s guidelines. The internal standard was created by pooling an aliquot of all biological samples in the experiment and labeling it with one of the Cy dyes (usually Cy2). Briefly, 50 μg of lysate was minimally labeled with 400 pmol Cy2, Cy3, and Cy5 and incubated on ice for at least 30 min in the dark. The labeling reaction was terminated by adding 1 μL 10 mM lysine and incubating the sample on ice for at least 15 min in the dark. Two samples labeled with Cy3 and Cy5 were analyzed on the same gel, together with a pooled sample as an internal standard, which was labeled with Cy2. Prior to isoelectric focusing (IEF), differentially labeled samples to be separated in the same gel were mixed and added to an equal volume of 2× sample buffer (7 M urea, 2 M urea, 4% CHAPS, 130 mM DTT, 2% Pharmalytes 3–10 NL) and ultimately brought to a total of 450 μL by addition of more of the samples dissolved in rehydration buffer (8 M urea, 2% CHAPS, 0.5% Pharmalytes 3–10 NL, 20 mM DTT). 2-DE was performed with Amersham Biosciences (Uppsala, Sweden) IPGphor IEF and Ettan Dalt Twelve
electrophoresis units. Precast IPG strips (24 cm, pH 3–10 NL) were used for the separation in the first dimension with a total focusing time of 76 kVh at 15°C. Prior to SDS-PAGE, each strip was equilibrated with 10 mL equilibration buffer A (6 M urea, 50 mM Tris-HCl, pH 8.8, 30% glycerol, 2% SDS, 10 mg/mL DTT) on a rocking table for 15 min, followed by equilibration with 10 mL equilibration buffer B (6 M urea, 50 mM Tris-HCl, pH 8.8, 30% glycerol, 2% SDS, 25 mg/mL iodoacetamide) for another 15 min. The strips were then loaded and run on 12.5% acrylamide gels. The running parameters were a constant power of 15 mA per gel at 15°C for 1 hour, 25 mA per gel at 15°C for 6 hours, and then 30 mA per gel at 15°C until the bromophenol blue dye front had run off the bottom of the gels. Labeled proteins were visualized with the Typhoon™ 9410 imager (GE Healthcare). All gels were scanned at 100 nm resolution, and the intensity was adjusted to ensure the maximum volume of each image was within 60,000–90,000 U. Images were cropped to remove areas extraneous to the gel image using ImageQuant V5.2 (Amersham Biosciences, UK) prior to analysis. Gel analysis was performed with DeCyder™ 6.5 (GE Healthcare). Sets of gels were first analyzed, and spots were counted using the differential in-gel analysis (DIA) mode of the DeCyder (GE Healthcare) software package, followed by a comprehensive biological variance analysis (BVA).

**In-gel digestion and MS/MS analysis**

Gels were fixed and stained with Coomassie brilliant blue. Proteins of interest, as defined by the 2D-DIGE/DeCyder analysis, were excised from the gels and digested by a modified in-gel tryptic digestion procedure. Briefly, gel pieces were washed in 50% ACN and 25 mM NH₄HCO₃ and then reduced with 10 mM DTT at 37°C and alkylated in the dark with 50 mM iodoacetamide (C₂H₄INO) at room temperature for 1 hour. After vacuum drying, the gel pieces were incubated with sequencing-grade modified trypsin at a concentration of 0.01 mg/mL in 25 mM NH₄HCO₃ for 16 hours at 37°C. Tryptic peptide mixtures were first extracted with 20 μL 5% TFA at 40°C for 1 hour and then re-extracted with the same volume of 2.5% TFA/50% ACN at 30°C for another 1
hour. The extracted solutions were blended, lyophilized, and used for identification by MALDI-TOF/TOF: peptide extracts were dissolved in 4 μL saturated matrix (7 mg/ml CHCA in 0.5% v/v TFA and 50% v/v ACN), and 0.6 μL of the mixture was spotted manually onto an ABI MALDI target plate. The spots were allowed to dry and then put into an ABI 4800 Proteomics Analyzer (Applied Biosystems, Framingham, MA, USA) equipped with a 200 Hz frequency-tripled Nd:YAG laser, operating at a wavelength of 355 nm and a repetition rate of 200 Hz in both MS and MS/MS modes. The laser intensity was set at 4300, and ions were collected between 700 Da and 4000 Da. All of the acquired MS spectra represented signal averaging of 1050 laser shots. The five most intense peptide spots with S/N exceeding 100 were selected and subjected to MS/MS analysis. MS/MS spectra were searched against the IPI rat database (v4.33) by GPS Explorer Software v2.0 (with MASCOT as the database search engine). The following search criteria were used: trypsin specificity, cysteine carbamidomethylation (C), and methionine oxidation (M) as variable modifications, 2 trypsin miscleavages allowed, 50 ppm MS tolerance, and 0.5 Da MS/MS tolerance.

**Western blot analysis**

Ten micrograms of protein were separated on 10% polyacrylamide gels and transferred to PVDF membranes (Amersham Pharmacia Biotech). Blots were blocked with 5% nonfat milk in TBS buffer with 0.1% Tween 20 (TBST) and then developed with diluted antibodies: anti-apolipoprotein monoclonal antibody (diluted 1:1000, Abcam, Cambridge, UK), anti-malate dehydrogenase antibody (diluted 1:1000, Abcam, Cambridge, UK), anti-pyruvate dehydrogenase antibody (diluted 1:1000, Santa Cruz Biotechnology, Dallas, TX, USA) and anti-GAPDH antibody (diluted 1:1000, Santa Cruz Biotechnology, Dallas, TX, USA) at 4°C overnight, followed by incubation with HRP-conjugated secondary antibodies for one hour prior to visualization of the bands with ECL reagents (Santa Cruz Biotechnology, Dallas, TX, USA)\textsuperscript{17}. All of the membranes were exposed to X-ray film and scanned with a GS-710 scanner (Bio-Rad).
Statistical analyses

Data are expressed as means ± SEM. The statistical significance of differences was assessed by Student’s t-tests, and values of p<0.05 were considered statistically significant.

Results

Effect of high-fat diet on rats

Adult rats were sacrificed after feeding them a high-fat diet for 8 weeks. The livers were rapidly excised and washed three times with PBS. Images were taken, and liver sections were stained with H&E. As shown in Fig. 1, after 6–8 weeks of HFD treatment, fatty liver disease symptoms were significantly observed. We found that the liver became yellow after 8 weeks of HFD feeding compared with the liver in the normal group, and the yellow liver atrophied and became brittle (Fig. 1A). HE staining showed that most of the hepatic lobules lost their normal architecture and morphology after 6-8 weeks of HFD treatment, while no histological abnormalities were observed in the control group (Fig. 1B). At 6 weeks of HFD treatment, fat droplets were significantly increased, with mild to moderate infiltration of inflammatory cells. By 8 weeks, most of the hepatic lobules had lost their normal structure, and a large number of macrovesicular droplets appeared (Fig. 1B). Routine blood testing showed that the levels of ALT, AST, and cholesterol were markedly increased and that leptin was significantly decreased after 4 weeks of HFD treatment. However, the HFD had no effect on body weight, TG, or blood glucose levels (Table 1). Together, these data indicate that hepatic steatosis and steatohepatitis were successfully induced by the HFD.

The fluorescent 2D-DIGE analysis

The major goal of this study was to determine the dynamic changes in the rat liver proteome during feeding with a high-fat diet. To this end, liver samples from rats fed the high-fat diet for 0, 2, 4, 6, and 8 weeks were collected and subjected to 2D-DIGE. The SDS-PAGE gels were stained and analyzed with the DeCyder™ 6.5 software (GE Healthcare). As shown in Fig. 2A, the protein...
spots in the gels were similar and widely distributed, and there were no obvious interference stripes or protein gathering phenomena. Image analysis showed that an average of 1626 (1577±49, n=6 Cy2 images) protein spots were detected (Fig. 2A and B). Then, the BVA module of the DeCyder™6.5 software was used to analyze the protein spot changes at each time point. According to the ANOVA test, there were 27 spots that exhibited statistically significant dynamic expression changes across all five experimental time points (1-ANOVA, p<0.05) (Fig. 2B). The dynamic changes in the protein spots in the five experimental groups were determined by BVA according to the ratios of log sample/standard. Twenty-seven protein spots exhibited significant up- or downregulated expression over time. The dynamic change profiles of the partial spots are shown in Fig. 3.

**Identification of differentially expressed proteins**

These 27 differentially expressed protein spots were picked from the preparative gels, digested by trypsin, and analyzed by mass spectrometry (MALDI-TOF/TOF MS/MS). The obtained protein PMF and MS/MS data were submitted to the Rat IPI 4.33 database. Finally, 24 proteins were successfully identified (Supplemental Table S1, Supplemental data). One such protein was spot 534, which was identified as the glucose-regulated protein precursor through Mascot analysis; the matching peptides were ITPSYVAFTPEGER, VTHAVVTVPAYFNDAQR, DNHLLGTFDLTGIPPAKR, and SDIDEIVLVGGSTR (Fig. 2C).

**Annotation of differentially expressed proteins**

To further understand the functions of the differentially expressed proteins, gene ontology annotation was performed by using the GOfact tool (http://61.50.138.118/gofact/). As shown in Fig. 4, each protein was linked to at least one Gene Ontology (GO) annotation category. Among them, 22 proteins were assigned to biological processes, 21 proteins were mapped to cellular components, and 24 proteins were involved in molecular function ontology. We found that most of the proteins were located in the mitochondria and extracellular matrix, among which were 5
proteins located in the mitochondria and 5 proteins annotated as extracellular matrix proteins; the other proteins were located in the endoplasmic reticulum, cytoskeleton, and microorganella (Fig. 4A). For biological processes, 14 proteins were identified as metabolism-related proteins, among which were 3 proteins involved with carbohydrates, 3 proteins related to lipid metabolism, 3 proteins involved in nucleic acid metabolism, and 5 proteins involved in amino acid metabolism; 4 proteins were related to biosynthesis; 2 proteins were involved in the response to stress; 2 proteins were related to cell communication; 2 proteins were related to development; and the other 5 proteins were involved in intracellular substance transfers (Fig. 4B). In the molecular function category, 3 GO terms in the binding and catalytic activity groups were enriched. Binding proteins were the major subcategory, including ion binding, protein binding, lipid binding, and nucleic binding (Fig. 4C).

**Protein validation by western blot**

Twenty-four proteins with a dynamic change profile were identified in the rats fed the HFD. To verify these DIGE results, protein samples from these experiments were further analyzed by western blot. As shown in Fig. 5, the 2D and 3D dynamic change maps of four protein spots changed in response to the high-fat diet for 8 weeks, and the western blotting results confirmed these results; we found that apolipoprotein A-I precursor and pyruvate dehydrogenase E1 were significantly increased after 8 weeks of HFD feeding. However, the expression levels of malate dehydrogenase and transthyretin precursor were downregulated.

**Discussion**

NAFLD is now considered to be the most common liver disease around the world, and the disease can progress slowly from simple nonalcoholic steatosis (NAS) to nonalcoholic steatohepatitis (NASH) and subsequently to hepatic fibrosis, cirrhosis of the liver, and hepatoma18, 19; however, the molecular mechanisms underlying NAFLD initiation and progression remain poorly understood. Proteomics is widely used to study the dynamic changes of proteins in tissues
and organs under physiological or pathological conditions. For instance, You et al. analyzed the protein profile and function of liver mitochondria from rats with nonalcoholic steatohepatitis by using a proteomic strategy. Using an LC-MS/MS combined label-free quantitative strategy, Rao et al. analyzed the high-density lipoprotein particles in patients with nonalcoholic fatty liver disease. Spanos et al. found by using iTRAQ combined with a nano-LC-MS/MS assay that dysregulation of GLO1 implicates the acetylation-ubiquitination degradation pathway in nonalcoholic fatty liver disease. In the present study, 2D-DIGE technology was used to systematically analyze the liver proteome during NAFLD in the HFD-induced rat, and the results will be helpful in elucidating the mechanisms involved in the progression of NAFLD.

Twenty-seven protein spots with dynamic changes were successfully observed in the present study, among which were 24 proteins identified by mass spectrometry. The gene ontology analysis showed that most of these proteins are metabolism-related proteins, including carbohydrate metabolism-related proteins (such as pyruvate dehydrogenase E1 component subunit beta, Pdhb; aldose 1-epimerase, Galm; malate dehydrogenase, Mdhl; and triosephosphate isomerase, Tpi1), lipid metabolism-related proteins (apolipoprotein A-I precursor, Apoal; 3-oxo-5-beta-steroid 4-dehydrogenase, as known as aldo-keto reductase family 1 member D1, Akr1D1), and amino acid and derivative metabolism-related proteins. Pdhb and Mdhl are two important enzymes in the TCA cycle. Pdhb participates in the transformation of pyruvic acid into acetyl CoA, and Mdhl catalyzes malic acid into oxaloacetic acid. We found that the expression of Pdhb was significantly increased in the rat liver after 8 weeks of HFD induction. Interestingly, the expression of Mdhl was dramatically decreased during the HFD-induction process. The upregulation of Pdhb suggested the acetyl CoA content increased during the HFD-induced process; however, the downregulation of Mdhl restrained the transformation of malic acid into oxaloacetic acid, which led to the TCA cycle pathway being suppressed, which resulted in a disorder of energy metabolism in liver cells.
Apolipoprotein is mainly synthesized in the liver and small bowel. ApoAI is the chief component of high-density lipoprotein (HDL)\textsuperscript{29}. It is well known that ApoAI is an especially important factor in the cholesterol reverse transportation process, and it can be combined with lecithin, a variety of plasma factors, and cell membrane receptors, as well as participate in plasma protein secretion and subsequently regulate HDL metabolism\textsuperscript{30}. In this study, we found that the expression of ApoAI was decreased in the early stages of HFD induction (0–4 weeks) and then increased in the later stages (6–8 weeks). It has been generally thought that a decline of ApoAI is related to viral hepatitis, liver cirrhosis, and liver cancer. However, an increase in ApoAI was found to be closely related to alcoholic hepatitis and high lipoprotein-α (Lp-α). This result indicates that there may be a different mechanism between high fat diet-induced liver tissue lesions and viral infection that leads to liver lesions.

Akr1D1 is an oxidoreductase that participates in bile acid synthesis and corticosteroid metabolism\textsuperscript{31-33}. In this study, we found that the expression of Akr1D1 was increased during the first 6 weeks and then significantly decreased at the 8-week point. Many studies have shown that liver bile acid is one of the important markers of anomalous changes during liver damage. Collectively, our data suggest that a distinct change of Akr1D1 in liver tissue indicates that a subclinical hepatic injury may be occurring.

Many publications have shown that the most important pathogenesis of fatty hepatitis is free radical-mediated liver damage, which mainly manifests as an oxidation product increase and/or a reduction in antioxidant effect\textsuperscript{34, 35}. It is well known that during the process of final pathogenesis of NAFLD, ROS are created, which would initiate an oxygen stress response and lead to lipid peroxidation and liver antioxidant system abnormalities\textsuperscript{36}. In this study, we found that catalase, which is related to hydrogen peroxide metabolism, increased and reached a peak at 4 weeks and then significantly decreased. The dramatic change in catalase levels shows that there was an
adjustment mechanism for high-fat diet processing in rats, which is consistent with previous reports\textsuperscript{37, 38}.

In summary, we adopted DIGE technology to monitor the dynamic change of proteins during the fatty liver formation process and found that 27 protein spots had dynamic changes, among which were 24 successfully identified proteins. GO annotation indicated that these proteins were implicated in the metabolism of carbohydrates, lipids, and amino acids. At present, the relationship between the changes in the expression of these proteins and the formation of fatty liver is not very clear. An in-depth study of these proteins will provide meaningful clues about the pathogenesis of fatty liver and early prevention and diagnosis.

**Abbreviations:**

2D-DIGE, two-dimensional difference gel electrophoresis; DMF, dimethyl formamide; IEF, isoelectric focusing; DTT, dithiothreitol; CHAPS, 3-[(3-cholamidopropyl) dimethylammonio] propanesulfonate; CHC, α-cyano-4-hydroxycinnamic acid; TFA, trifluoroacetic acid; IPI, international protein index; NAFLD, nonalcoholic fatty liver disease; DIA, differential in-gel analysis; BVA, biological variance analysis; CBB, Coomassie brilliant blue; TBST, Tris-buffered saline Tween-20; HRP, horseradish peroxidase; MALDI-TOF/TOF, matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometry; TCA, tricarboxylic acid cycle

**Acknowledgments**

This work was supported by grants from the Chinese Natural Science Foundation (No. 31660242, 81770310), the Guangxi Natural Science Foundation (No. 2016GXNSFBC380001, 2017GXNSFFA198003), and the Key State Laboratory Talent Project (No. CMEMR2016-A01).
References
[1] Chen M, Ying W, Song Y, Liu X, Yang B, Wu S, Jiang Y, Cai Y, He F, Qian X: Analysis of human liver proteome using replicate shotgun strategy. Proteomics 2007, 7:2479-88.
[2] Beretta L: HUPO's effort to define the liver proteome. Journal of proteome research 2010, 9:637-8.
[3] Davis GL, Keeffe EB: Advances in liver disease. Highlights from the 51st Annual Meeting of the American Association for the Study of Liver Diseases, October 27-31, 2000, Dallas, TX. Reviews in gastroenterological disorders 2001, 1:42-7.
[4] Jacobson IM, Davis GL, El-Serag H, Negro F, Trepo C: Prevalence and challenges of liver diseases in patients with chronic hepatitis C virus infection. Clinical gastroenterology and hepatology: the official clinical practice journal of the American Gastroenterological Association 2010, 8:924-33; quiz e117.
[5] Bernal Reyes R: [Liver diseases. Non-alcoholic fatty liver disease]. Revista de gastroenterologia de Mexico 2010, 75 Suppl 1:181-3.
[6] Mikolasevic I, Milic S, Racki S, Zaputovic L, Stimac D, Radic M, Markic D, Orlic L: Nonalcoholic Fatty Liver Disease (NAFLD)-a New Cardiovascular Risk Factor in Peritoneal Dialysis Patients. Peritoneal dialysis international: journal of the International Society for Peritoneal Dialysis 2015.
[7] Marrero JA, Fontana RJ, Su GL, Conjeevaram HS, Emick DM, Lok AS: NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States. Hepatology 2002, 36:1349-54.
[8] Younossi ZM, Otgonsuren M, Henry L, Venkatesan C, Mishra A, Erario M, Hunt S: Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. Hepatology 2015.
[9] Allard JP: Other disease associations with non-alcoholic fatty liver disease (NAFLD). Best practice & research Clinical gastroenterology 2002, 16:783-95.
[10] Almobarak AO, Barakat S, Khalifa MH, Elhoweris MH, Elhassan TM, Ahmed MH: Non alcoholic fatty liver disease (NAFLD) in a Sudanese population: What is the prevalence and risk factors? Arab journal of gastroenterology: the official publication of the Pan-Arab Association of Gastroenterology 2014, 15:12-5.
[11] Adams LA, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, Angulo P: The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology 2005, 129:113-21.
[12] Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ: Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999, 116:1413-9.
[13] Tannu NS, Hemby SE: Two-dimensional fluorescence difference gel electrophoresis for comparative proteomics profiling. Nat Protoc 2006, 1:1732-42.
[14] Qian WJ, Jacobs JM, Liu T, Camp DG, 2nd, Smith RD: Advances and challenges in liquid chromatography-mass spectrometry-based proteomics profiling for clinical applications. Mol Cell Proteomics 2006, 5:1727-44.
[15] Gharbi S, Gaffney P, Yang A, Zvelebil MJ, Cramer R, Waterfield MD, Timms JF: Evaluation of two-dimensional differential gel electrophoresis for proteomic expression analysis of a model breast cancer cell system. Mol Cell Proteomics 2002, 1:91-8.
[16] Zhang X, Yang J, Guo Y, Ye H, Yu C, Xu C, Xu L, Wu S, Sun W, Wei H, Gao X, Zhu Y, Qian X, Jiang Y, Li Y, He F: Functional proteomic analysis of nonalcoholic fatty liver disease in rat models: enoyl-coenzyme a hydratase down-regulation exacerbates hepatic steatosis. Hepatology 2010, 51:1190-9.
[17] Chen M, Wang Y, Zhao Y, Wang L, Gong J, Wu L, Gao X, Yang Z, Qian L: Dynamic proteomic and metabonomic analysis reveal dysfunction and subclinical injury in rat liver during restraint stress. Biochimica et biophysica acta 2009, 1794:1751-65.
[18] Feldstein AE, Werneburg NW, Canbay A, Guicciardi ME, Bronk SF, Rydzewski R, Burgart LJ, Gores GJ: Free fatty acids promote hepatic lipotoxicity by stimulating TNF-alpha expression via a lysosomal pathway. Hepatology 2004, 40:185-94.
[19] Schmocker C, Weylandt KH, Kahlke L, Wang J, Lobeck H, Tieg S, Berg T, Kang JX: Omega-3 fatty acids alleviate chemically induced acute hepatitis by suppression of cytokines. Hepatology 2007, 45:864-9.
[20] Bell LN, Theodorakis JL, Vuppalanchi R, Saxena R, Bemis KG, Wang M, Chalasani N: Serum proteomics and biomarker discovery across the spectrum of nonalcoholic fatty liver disease. Hepatology 2010, 51:111-20.
[21] Yilmaz Y: Serum proteomics for biomarker discovery in nonalcoholic fatty liver disease. Clinica chimica acta; international journal of clinical chemistry 2012, 413:1190-3.
[22] Nuno-Lambarri N, Barbero-Becerra VJ, Uribe M, Chavez-Tapia NC: Mitochondrial Molecular Pathophysiology of Nonalcoholic Fatty Liver Disease: A Proteomics Approach. International journal of molecular sciences 2016, 17:281.
[23] Tsai MT, Chen YJ, Chen CY, Tsai MH, Han CL, Chen YJ, Mersmann HJ, Ding ST: Identification of Potential Plasma Biomarkers for Nonalcoholic Fatty Liver Disease by Integrating Transcriptomics and Proteomics in Laying Hens. The Journal of nutrition 2017, 147:293-303.
[24] You Y, Zhang Y, Lu Y, Hu K, Qu X, Liu Y, Lu B, Jin L: Protein profiling and functional analysis of liver mitochondria from rats with nonalcoholic steatohepatitis. Molecular medicine reports 2017, 16:2379-88.
[25] Rao PK, Merath K, Drigalenko E, Jadhav AYL, Komorowski RA, Goldblatt MI, Rohatgi A, Sarzynski MA, Gawrieh S, Olivier M: Proteomic characterization of high-density lipoprotein particles in patients with non-alcoholic fatty liver disease. Clinical proteomics 2018, 15:10.
[26] Spanos C, Maldonado EM, Fisher CP, Leenutaphong P, Oviedo-Orta E, Windridge D, Salguero FJ, Bermudez-Fajardo A, Weeks ME, Evans C, Corfe BM, Rabbani N, Thornalley PJ, Miller MH, Wang H, Dillon JF, Quaglia A, Dhawan A, Fitzpatrick E, Bernadette Moore J: Proteomic identification and characterization of hepatic glyoxalase I dysregulation in non-alcoholic fatty liver disease. Proteome science 2018, 16:4.
[27] Patel MS, Korotchkina LG, Sidhu S: Interaction of E1 and E3 components with the core proteins of the human pyruvate dehydrogenase complex. J Mol Catal B Enzym 2009, 61:2-6.
[28] Kim SY, Lee PY, Shin HJ, Kim do H, Kang S, Moon HB, Kang SW, Kim JM, Park SG, Park BC, Yu DY, Bae KH, Lee SC: Proteomic analysis of liver tissue from HBx-transgenic mice at early stages of hepatocarcinogenesis. Proteomics 2009, 9:5056-66.
[29] Aldred S, Sozzi T, Mudway I, Grant MM, Neubert H, Kelly FJ, Griffiths HR: Alpha tocopherol supplementation elevates plasma apolipoprotein A1 isoforms in normal healthy subjects. Proteomics 2006, 6:1695-703.
[30] Ogasawara K, Mashiba S, Hashimoto H, Kojima S, Matsuno S, Takeya M, Uchida K, Yajima J: Low-density lipoprotein (LDL), which includes apolipoprotein A-I (apoAI-LDL) as a novel marker of coronary artery disease. Clin Chim Acta 2008, 397:42-7.
[31] Jiang J, Nilsson-Ehle P, Xu N: Influence of liver cancer on lipid and lipoprotein metabolism. Lipids Health Dis 2006, 5:4.
[32] Di Costanzo L, Drury JE, Christianson DW, Penning TM: Structure and catalytic mechanism of human steroid Sbeta-reductase (AKR1D1). Mol Cell Endocrinol 2009, 301:191-8.
[33] Lee WH, Lukacik P, Guo K, Ugochukuwu E, Kavanagh KL, Marsden B, Oppermann U: Structure-activity relationships of human AKR-type oxidoreductases involved in bile acid synthesis: AKR1D1 and AKR1C4. Mol Cell Endocrinol 2009, 301:199-204.
Kumar A, Sharma A, Duseja A, Das A, Dhiman RK, Chawla YK, Kohli KK, Bhansali A: Patients with Nonalcoholic Fatty Liver Disease (NAFLD) have Higher Oxidative Stress in Comparison to Chronic Viral Hepatitis. Journal of clinical and experimental hepatology 2013, 3:12-8.

Madan K, Bhardwaj P, Thareja S, Gupta SD, Saraya A: Oxidant stress and antioxidant status among patients with nonalcoholic fatty liver disease (NAFLD). Journal of clinical gastroenterology 2006, 40:930-5.

Suh SK, Hood BL, Kim BJ, Conrads TP, Veenstra TD, Song BJ: Identification of oxidized mitochondrial proteins in alcohol-exposed human hepatoma cells and mouse liver. Proteomics 2004, 4:3401-12.

Walsh K, Alexander G: Alcoholic liver disease. Postgrad Med J 2000, 76:280-6.

Angulo P: Nonalcoholic fatty liver disease. N Engl J Med 2002, 346:1221-31.
Figure Legends

**Fig. 1. Effect of HFD on liver hepatic pathology.** A, Photograph of rat liver tissue (i-iii, control group; iv-vi, HFD induction for 8 weeks). B, Representative images of hematoxylin and eosin-stained liver from rats fed a high-fat diet for different periods are shown (×200).

**Fig. 2. DIGE images of differentially expressed protein spots.** A, Two-dimensional DIGE images of the control and HFD-induced rats for 0 weeks, 2 weeks, 4 weeks, 6 weeks, and 8 weeks. B, Protein spots with significant changes were labeled and identified. Spot numbers correspond to those in supplemental data Table S1. pH 3–10, 300 μg proteins were loaded. C, The sequences of the precursor at m/z 1566.84, 1888.04, and 1943.08 were analyzed by MS/MS and found to be ITPSYVAFTEGER, VTHAVVTVPAYFNDAPQR, and DNHLLGTFDLTGIAPPAPR. This protein was identified to be the glucose-regulated protein precursor after a database search.

**Fig. 3. The dynamic profiles of some key protein spots.** Graphical representations of partial protein spots that showed dynamic changes during the 8 weeks of HFD induction; the left panel shows the images of the spots in the 2D gel. The volume of each spot was calculated and normalized by the Decyder-DIA software (right panel). Values are indicated as the standardized log of abundance.

**Fig. 4. Gene ontology annotation of identified proteins.** Identified proteins were categorized based upon their subcellular location (A), biological process (B), and molecular function (C). Values in these pie charts represent the number of proteins found in that respective category for all submitted proteins with GO annotation.
Fig. 5. Protein quantitative confirmation with western blotting. The two-dimensional and three-dimensional fluorescence intensity profiles of spots 1572, 1280, 1353, and 1708 at the 0- and 8-week time points are shown. These proteins and the changes in expression were validated by western blot.
Fig. 1. Effect of HFD on liver hepatic pathology. A, Photograph of rat liver tissue (i-iii, control group; iv-vi, HFD induction for 8 weeks). B, Representative images of hematoxylin and eosin-stained liver from rats fed a high-fat diet for different periods are shown (×200).
Fig. 2. DIGE images of differentially expressed protein spots. A, Two-dimensional DIGE images of the control and HFD-induced rats for 0 weeks, 2 weeks, 4 weeks, 6 weeks, and 8 weeks. B, Protein spots with significant changes were labeled and identified. Spot numbers correspond to those in supplemental data Table S1. pH 3–10, 300 μg proteins were loaded. C, The sequences of the precursor at m/z 1566.84, 1888.04, and 1943.08 were analyzed by MS/MS and found to be ITPSYVAFTPEGER, VTHAVVTVPAYFNDAQR, and DNHLLGTFDLTGIPPAKR. This protein was identified to be the glucose-regulated protein precursor after a database search.
Fig. 3. The dynamic profiles of some key protein spots. Graphical representations of partial protein spots that showed dynamic changes during the 8 weeks of HFD induction; the left panel shows the images of the spots in the 2D gel. The volume of each spot was calculated and normalized by the Decyder-DIA software (right panel). Values are indicated as the standardized log of abundance.
**Fig. 4. Gene ontology annotation of identified proteins.** Identified proteins were categorized based upon their subcellular location (A), biological process (B), and molecular function (C). Values in these pie charts represent the number of proteins found in that respective category for all submitted proteins with GO annotation.
Fig. 5. Protein quantitative confirmation with western blotting. The two-dimensional and three-dimensional fluorescence intensity profiles of spots 1572, 1280, 1353, and 1708 at the 0- and 8-week time points are shown. These proteins and the changes in expression were validated by western blot.
Table 1. Biochemical parameters in the different groups

| Parameter          | 0 w (n=8) | 2 w (n=8) | 4 w (n=8) | 6 w (n=8) | 8 w (n=8) |
|--------------------|-----------|-----------|-----------|-----------|-----------|
|                    | CTL       | HFD       | CTL       | HFD       | CTL       | HFD       | CTL       | HFD       | CTL       | HFD       |
| Body weight (g)    | 241.5±20.2| 254.4±17.1| 267.5±25.4| 286.3±21.8| 320.2±25.1| 317.5±24.3| 345.2±23.5| 354.2±26.0| 379.5±39.4| 387.9±33.6|
| TG (mmol/L)        | 0.28±0.15 | 0.30±0.17 | 0.30±0.16 | 0.31±0.23 | 0.35±0.14 | 0.37±0.19 | 0.38±0.08  | 0.35±0.14  | 0.35±0.14  | 0.41±0.23  | 0.50±0.25  |
| Cholesterol (mmol/L)| 1.26±0.41 | 1.13±0.33 | 1.16±0.21 | 1.01±0.24 | 1.42±0.26 | 1.55±0.18 | 1.53±0.14 | 2.53±0.29* | 1.69±0.14  | 2.61±0.32* |
| ALT (U/L)          | 27.2±4.6  | 25.2±5.4  | 31.6±16.2 | 34.7±15.7 | 36.5±18.1 | 67.3±20.1**| 45.2±21.4 | 132.2±30.5**| 43.4±32.5  | 211.4±33.7**|
| AST (U/L)          | 130.8±19.8| 137.8±24.6| 143.2±32.5| 151.2±37.3| 142.54±26.3| 170.69±35.1*| 145.1±16.55| 257.4±26.9**| 137.6±21.2 | 297.6±36.3**|
| Glucose (mg/dL)    | 5.1±1.76  | 4.5±0.75  | 4.9±1.55  | 5.2±1.36  | 5.7±1.04  | 5.5±1.2  | 5.16±0.53 | 4.94±0.93  | 5.05±1.02  | 4.79±1.17  |
| Leptin (ng/dL)     | 0.61±0.09 | 0.73±0.21 | 0.63±0.07 | 0.67±0.08 | 0.75±0.05 | 0.42±0.11*| 0.86±0.11 | 0.57±0.15* | 0.93±0.15  | 0.54±0.24*  |

Data represent the means ± SEM. *p<0.05, HFD versus CTL; **p<0.01, HFD versus CTL.
| Spot No | IPI No | Protein description | P-Value ID | Mr (Da) | PI (%) | Score | Peptide Hits | Peptides identified |
|---------|--------|---------------------|------------|---------|--------|--------|---------------|---------------------|
| 1572    | IPI00197703 | Apolipoprotein A-I precursor | 4E-23      | 30043   | 5.52   | 71%    | 270           | 4 K.VVAEEFR.D, K.FGLYSDQMR.E, K.WNEEVEAYR.Q, K.DSGRDYVSQFESSTLQK.Q |
| 1271    | IPI00210823 | 3-oxo-5-beta-steroid dehydrogenase | 4-6.4E-46  | 37354   | 6.18   | 54%    | 498           | 4 K.SLGVSNFNR.R, R.HIDGAYVYRN, R.IKENFQIFDFSRLNK.Q, K.YKPVTNQVEC*HPYFTQTK.L |
| 1510    | IPI00231767 | Triosephosphate isomerase | 8.00E-25   | 26832   | 6.89   | 75%    | 287           | 2 K.DLGATWVVLGHSER.R, K.LPADTEVVC*APPTAYIDFAR.Q |

**Carbohydrate metabolism**

| Spot No | IPI No | Protein description | P-Value ID | Mr (Da) | PI (%) | Score | Peptide Hits | Peptides identified |
|---------|--------|---------------------|------------|---------|--------|--------|---------------|---------------------|
| 1280    | IPI00198717 | Malate dehydrogenase, cytoplasmic | 6.4E-39    | 36460   | 6.16   | 46%    | 428           | 5 K.ENFSC*LTR.L, K.GEFITTVQQR.G, K.DLDVAVLVGSMPR.R, K.FVEGLPINDFSR.E, K.SAPSPKENFSC*LTR.L |
| 1121    | IPI00360056 | Aldose 1-epimerase | 1.30E-11   | 37869   | 6.18   | 42%    | 155           | 3 K.EYHLPINR.E, K.QPYFGAVVGR.V, R.HLQSYYIHGFDHNFCLK |
| 1353    | IPI00194324 | Pyruvate dehydrogenase component subunit beta E1 | 3.2E-11    | 38957   | 6.2    | 34%    | 151           | 2 R.IMEGPAFNLIPAVR.V, K.TYYMSAGLQPVPIVFR.G |
| Accession  | Description                                      | Description                  | E-value | FDR   | PEAKS | Identity | Length |
|------------|--------------------------------------------------|-------------------------------|---------|-------|-------|----------|--------|
| 1273       | Translation elongation factor 1-                  | delta subunit (Fragment)     | 2.00E-14| 4.86  | 49%   | 183      | 3      |
| 502        | Kininogen 1                                       |                               | 3.20E-20| 6.29  | 32%   | 241      | 3      |
| 37         | Carbamoyl-phosphate synthase, mitochondrial       | precursor                    | 5.0E-14| 6.33  | 22%   | 179      | 4      |
| 1743       | D-dopachrome decarboxylase                        |                               | 1.0E-33| 6.09  | 79%   | 376      | 5      |
| 1708       | Transthyretin precursor                           |                               | 1.0E-10| 5.77  | 20%   | 146      | 1      |
| 1168       | Arginase-1                                        |                               | 1.6E-29| 6.51  | 62%   | 334      | 3      |
| 564        | Delta-1-pyrroline-5-carboxylate dehydrogenase    |                               | 1.30E-08| 7.14  | 26%   | 125      | 2      |
| 1400       | Agmatinase, mitochondrial                         | precursor                    | 4.00E-25| 6.71  | 32%   | 290      | 4      |
| Gene ID     | Protein Name                                      | Change | Fold Change | P Value | Q Value | Expression | Kmers                                                                 |
|-------------|--------------------------------------------------|--------|-------------|---------|---------|------------|-----------------------------------------------------------------------|
| IPI00231742 | Catalase                                         | 0.0031 | 7.07        | 22%     |         | 71         | R.LGPonyQIPVNCpY.R.A                                                 |
| IPI00327469 | Alpha-2-HS-glycoprotein precursor                | 3.2E-06| 6.05        | 21%     |         | 101        | K.VGQPdAGAAGpVAPLCpG.R.V                                             |
| IPI00206624 | Glucose-regulated protein precursor              | 1.30E-40| 5.07        | 50%     |         | 445        | K.SDIDEIVLVggstr.I                                                   |
| IPI00230788 | Carbonic anhydrase 3                             | 6.40E-43| 6.89        | 85%     |         | 468        | R.VvFdddTfdr.S                                                        |
| IPI00389571 | Keratin, type II cytoskeletal 8                  | 1.3e-042| 5.83        | 55%     |         | 465        | K.LalDIEIATyr.K                                                        |
| IPI00561017 | Prdx5 22 kDa protein                             | 6.40E-45| 8.54        | 55%     |         | 488        | K.thLpgFveQagalk.k                                                    |
| IPI00760117 | Comt Isoform 2 of Catechol O-methyltransferase  | 2.00E-10| 5.11        | 55%     |         | 143        | R.LLQPGAR.L                                                           |

**Response to stimulus**

**Binding**

**Development**

**Unclassified**
| Spot No. | Accession | Protein Name                      | p-value ID  | Amino Acid Sequence Coverage | Score | Peptide Hits |
|---------|-----------|----------------------------------|-------------|-----------------------------|-------|--------------|
| 1563    | IPI00392676 | Bilverd predicted reductase B    | 2.00E-19    | 6.29 62%                    | 233   | 3            |
|         |           |                                  |             |                             |       |              |
| 1746    | IPI00205036 | Hemoglobin alpha 2 chain         | 3.20E-54    | 8.45 96%                    | 581   | 5            |
|         |           |                                  |             |                             |       |              |
| 1453    | IPI00231106 | 3-mercaptopyruvate sulfurtransferase | 1.3E-36    | 5.88 57%                    | 405   | 4            |

\(^a\) Spot No. is the unique number of the position of the spot in the master gel.

\(^b\) p-value ID is the best expectation value for the identified protein as calculated by Mascot.

\(^c\) Amino acid sequence coverage for the identified protein.

\(^d\) Score is the protein score based on combined mass and mass/mass spectrums.

\(^e\) Peptide hits is the unique number of MS/MS spectrums which match to the trypsin peptide.
| Sport ID | Max   | Anova  | Wilcoxon | ratio > 2 & Anova < 0.05 |
|---------|-------|--------|----------|--------------------------|
| 37      | 2.0789| 0.0303203 | 0        | 1 "1" "1" "1" "1" |
| 465     | 3.8932| 0.00104345 | 0        | 1 "1" "1" "1" "1" |
| 527     | -3.52332 | 0.0124988 | 0        | 1 "1" "1" "1" "1" |
| 543     | 2.87164| 0.00150523 | 0        | 1 "1" "1" "1" "1" |
| 559     | 2.26754| 0.023475 | 0        | 1 "1" "1" "1" "1" |
| 560     | 2.64349| 2.31345e-4 | 0        | 1 "1" "1" "1" "1" |
| 564     | 2.47844| 0.0463818 | 0        | 1 "1" "1" "1" "1" |
| 502     | 2.23441| 0.0148328 | 0        | 1 "1" "1" "1" "1" |
| 799     | 2.29926| 0.0166998 | 0        | 1 "1" "1" "1" "1" |
| 1121    | -2.39675| 0.0231339 | 0        | 1 "1" "1" "1" "1" |
| 1271    | 3.02366| 0.00121944 | 0        | 1 "1" "1" "1" "1" |
| 1273    | -2.4166| 0.0024406 | 0        | 1 "1" "1" "1" "1" |
Supplemental data illustrates the MALDI-TOF-TOF MS/MS spectra of ions from the proteins that listed in Table 2, and the detailed information of mascot search results.

**Spot 1572**
Match to: IPI00197703 Score: 270 Expect: 4e-023  
Tax_Id=10116 Gene_Symbol=Apoa1 Apolipoprotein A-I precursor  
Nominal mass (M_r): 30043; Calculated pI value: 5.52  
Sequence Coverage: 71%  
Matched peptides shown in Bold Red  

| 1280 | -2.2932 | 5.92003e-4 | 0 | 1 | "1" "1" "1" "1" |
| 1353 | 2.02762 | 0.00764313 | 0 | 1 | "1" "1" "1" "1" |
| 1400 | 2.20124 | 0.0123302 | 0 | 1 | "1" "1" "1" "1" |
| 1453 | -3.24803 | 2.03383e-4 | 0 | 1 | "1" "1" "1" "1" |
| 1510 | 2.10708 | 7.60294e-5 | 0 | 1 | "1" "1" "1" "1" |
| 1514 | 2.16199 | 0.0280563 | 0 | 1 | "1" "1" "1" "1" |
| 1526 | -2.97009 | 0.00582803 | 0 | 1 | "1" "1" "1" "1" |
| 1563 | -2.01115 | 0.0171667 | 0 | 1 | "1" "1" "1" "1" |
| 1572 | 2.52943 | 0.0121619 | 0 | 1 | "1" "1" "1" "1" |
| 1662 | 2.1056 | 0.00616959 | 0 | 1 | "1" "1" "1" "1" |
| 1685 | -2.13905 | 0.00174601 | 0 | 1 | "1" "1" "1" "1" |
| 1708 | -2.51956 | 0.0135997 | 0 | 1 | "1" "1" "1" "1" |
| 1743 | -2.14373 | 0.00749905 | 0 | 1 | "1" "1" "1" "1" |
| 1746 | -2.54728 | 0.0102367 | 0 | 1 | "1" "1" "1" "1" |
| 1754 | -3.50464 | 0.00260713 | 0 | 1 | "1" "1" "1" "1" |

Probability Based Mowse Score  
Ions score is -10*Log(P), where P is the probability that the observed match is a random event.  
Protein scores greater than 59 are significant (p<0.05).  
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.
MS/MS Fragmentation of **FGLYSDQMR**

MS/MS Fragmentation of **VVAEEFR**

MS/MS Fragmentation of **WNEEVEAYR**

MS/MS Fragmentation of **DSGRDYVSQFESSTLGK**
Spot 1271

Match to: IPI00210823 Score: 498 Expect: 6.4e-046

Tax_Id=10116 Gene_Symbol=Akr1d1 3-oxo-5-beta-steroid 4-dehydrogenase

Nominal mass (M_r): 37354; Calculated pI value: 6.18
Sequence Coverage: 54%

Matched peptides shown in **Bold Red**

1  MNLSTANHHI PLNDGNSIPI IGLGTYSDRP PVPGKTFIAV KTAIDEGYRH
51  IDGAYVYRE HEVGEAIREK VAEGVKREE IFYCGKLWST DHDPEMVRPA
101  LERTLQTTLK DYIDLHYIEM PMAFKPGEF YPKDENGYI YHKSNLATW
151  EALEACKDAG LVKSGLSVSNF NRRQLEVILN KGKLYKPTQ NQVECHPYFT
201  QTKLLEVSAS SMSTFIVAYS PLGTGLNPLW VNVSSPPLLK DELLTSLLKK
251  YNKTQAQIVL RFDIQRGLLV IPKSTTPERI KENFQIFDFS LTKEEMKDEIE
301  ALNKVRFEV MLMSDIPEY PPFHDEY

**Probability Based Mowse Score**

Ions score is \(-10 \times \log(P)\), where P is the probability that the observed match is a random event.

Protein scores greater than 59 are significant (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.
MS/MS Fragmentation of YKPVTNQVECHPYFTQTK

MS/MS Fragmentation of SLGVSNFNR

MS/MS Fragmentation of HIDGAYVYR
Spot 1510
Match to: IPI00231767 Score: 287 Expect: 8e-025
Tax_Id=10116 Gene_Symbol=Tpi1 Triosephosphate isomerase
Nominal mass ($M_r$): 26832; Calculated pI value: 6.89
Sequence Coverage: 75%
Matched peptides shown in Bold Red

| Start | End | Sequence |
|-------|-----|----------|
| 1     | 51  | MAPSRKFFVG GNWKMGRRK CLGELICTLN AAKLPADTEV VCAPPTAYID |
| 51    | 101 | FARQKLPKIAVAAQCYKV TNGAFTGEIS PGMKIDLGAT WVLGHSERR |
| 101   | 151 | HIFGESDELIGQKVNHALSE GLGVIACIGELDEREAGIT EKVVFQTKA |
| 151   | 201 | TADNYKDWCK VVLAYEPVWA IGGTKATPQ QAQEVHEKLRL GWLKNVSEG |
| 201   | 251 | VACQCTRIYYGSVTGATCKE LASQPDVDFGLVGGASLKPE FVDIINAKQ |

Probability Based Mowse Score

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 59 are significant (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

MS/MS Fragmentation of DLGATWVLGHSER

MS/MS Fragmentation of LPADTEVCAPPTAYIDFAR
Spot 1280
Match to: IPI00198717 Score: 428 Expect: 6.4e-039
Tax_Id=10116 Gene_Symbol=Mdh1 Malate dehydrogenase, cytoplasmic
Nominal mass (M): 36460; Calculated pI value: 6.16
Sequence Coverage: 46%
Matched peptides shown in **Bold Red**

1 MSEPIRVLVT GAAGQIAYSL LYSIGNGSVF GKDIPIILVL LDITPMMGVL 51 DGVLMELQDC ALPLLDQVIA TDKHEETAFKD LDVAVLVGSM PRREGMERKD
101 LLKANVKIKF SQGAALEKYA KKSVKVIVVGPANTNCLTA SKSAPSIPKE 151 NFSCLTRDHL NRASKQIALK LGVTADDVKN VIWGNHSST QYPDVNHAKV
201 KLOGKEVGYV EALKDWSVWL GEFITTVQQR GAAVIKARKL SSAMSAAKAI 251 SDHIRD1WFG TPEGEFVSMG VISDGNSYGV PDDLILSFVP VIKNKTWFVF
301 EGLPINDFSR EKMDLTADEL TEKETAEFEF LSSA

**Probability Based Mowse Score**

Ions score is \(-10 \log(P)\), where P is the probability that the observed match is a random event.

Protein scores greater than 59 are significant (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

**MS/MS Fragmentation of FVEGLPINDFSR**

**MS/MS Fragmentation of GEFFITTVQQR**
Spot 1121
Match to: IPI00360056 Score: 325 Expect: 1.3e-028
Tax_Id=10116 Gene_Symbol=Galm Aldose 1-epimerase
Nominal mass (M): 37869; Calculated pI value: 6.18
Sequence Coverage: 51%
Matched peptides shown in Bold Red

1 MVSVTVFPG ELPSSGGAKE KFQLRSDQNL VDIISWGCTI TALQVKDRQG
51 KASDVLGFA ELEGYLKQYP YFGAVVGRVA NRIA KGRFTV DGKEYHLPIN
101 REPNSLHGCPPGRGFDK VMLWTP QVLSNGVQFS RVSPDGEEGY PGEKLVWVTY
151 TLDGELVYNN YRAASQTTP VNLTNHISYN LAGQGSPDIY DHEV TIAAD
201 YLPVDETLP TGVIAPVEGT AFDLKPVELGKHLQSYHIIHPGDHNFCFLK
251 SKEKFCARV HHAASGRIETYTTQPGVQF YTNFLDGTLLKJKGSGEVPK
301 HSGFCLETQY WPADVNQPFQPPILLRPGEE YNHTTWFKFSVA

Probability Based Mowse Score

Ions score is $-10 \log(P)$, where $P$ is the probability that the observed match is a random event.
Protein scores greater than 59 are significant ($p<0.05$).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

MS/MS Fragmentation of HLQSYHIIHGDHNFCFLK

MS/MS Fragmentation of VWVTYTLGDELVNVYR
MS/MS Fragmentation of **EYHLPINR**

MS/MS Fragmentation of **QPYFAGVGR**
**Spot 1353**

Match to: IPI00194324 Score: 151 Expect: 3.2e-011

Tax_Id=10116 Gene_Symbol=Pdhb Pyruvate dehydrogenase E1 component subunit beta, mitochondrial precursor

Nominal mass (M_r): 38957; Calculated pI value: 6.20

Sequence Coverage: 34%

Matched peptides shown in **Bold Red**

1. **MAAVGLVR** PLRQASGLLK RRFHRSAPAA VQLTVERAIN QGMDEELERD
2. **EKVFLLGEEV AQYDGAYKVS** RGLWWKKYDK RIIDTPISEM GFAIAVGAA
3. **MAGLRPICEF MTFNFSMQAI** DQVINSAAKT **YYMSAGLQPV PIVFR** GPNGA
4. **SAGVAQHSQ CFAAWYGHCP GLKVSPWNS EDAGGLKSA IRDDNPVVML**
5. **ENELMYGVAF ELIPTEAQS** FLIGAKI EROQTHITV AHSRPGHCL
6. **EAAVLSKEG IICEVINLR** IRPMDEIAE ASVMKTNHLV TVEGGWPQFG
7. **VGAECARIM EGPAFNFLDA PAVR** VTGADV PMPVAKILED NSIPQVKDII
8. **FAIKTLNI**

**Probability Based Mowse Score**

Ions score is $-10\log(P)$, where $P$ is the probability that the observed match is a random event.

Protein scores greater than 59 are significant ($p<0.05$).

Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

**MS/MS Fragmentation of IMEFGPAVNFLDAPAVR**

**MS/MS Fragmentation of TYYMSAGLQPVPIVFR**
Spot 1273
Match to: IPI00197900 Score: 183 Expect: 2e-014
Tax_Id=10116 Gene_Symbol=Eef1d Translation elongation factor 1-delta subunit
Nominal mass (M_r): 28748; Calculated pI value: 4.86
Sequence Coverage: 49%
Matched peptides shown in Bold Red

1 MATNFLMHEK IWFDKFKYDD AERFFYEQMN GPVTAGSRQS SGPGASSGPG
51 GDHSIDLVRRI ASLEVENQNL RGVVQLQQA ISKLEVRST LEKSSPHTRA
101 TAPQTQHVSP MRQVEPPAKK GATPAEDDED NDIDLFGSDE EEEDEEAARL
151 REERLRQYAE KKAKKPTLVA KSSILLDVKPV WDDETDAQLE ETCVRS1QLD
201 GLWVGASKLVL PVGYGIRKLQ IQCVDDEKVT GTDLLEEET KFEEHVQSVDD
251 IAAPNKI

Probability Based Mowse Score

Ions score is -10*Log(P), where P is the probability that the observed match is a
random event.
Protein scores greater than 59 are significant (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking
protein hits.

MS/MS Fragmentation of LVPVGYGIR
Spot 502
Match to: IPI00515829 Score: 191 Expect: 3.2e-015
Tax_Id=10116 Gene_Symbol=Kng1 Kininogen 1
Sequence Coverage: 37%
Matched peptides shown in Bold Red

1 MKLITILLLC SRLPLSQAEG AQELCNDETVFQAVDTALKKYNAELES
51 GNPQVLVRVT KGTKDGAETLYSFKVQIKEGNCVQGLTWQDCKDKDAE
101 EAATGECTITT LGKENKPSVATQICNITPGKGPKKTEEDL CVCFCQPIM
151 DSSDLKPVKHLVEHFPNNTKHTHLFAKESKAHSQVAGMNYKIIYSI
201 VQTNCKSEDFPSLREDCVPLPYGDGECTGHTHVDIHTNLGAQSGQCDLY
251 PGDDLFELLPNKNGCPRFI PDVDSEPKEALGHSIAQLN4QHHIPYPFK
301 DTVKKATSOV VAGVIYVIEF IAETNSQSKETLADCEKHQGQLNC
351 NADVYMRPWE NKVPTVRCQALDMISRPPGFSPFLVRVQETKEGTRL
Probability Based Mouse Score

Ions score is $-10\times\log(P)$, where $P$ is the probability that the observed match is a random event. Protein scores greater than 59 are significant (p<0.05). Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

MS/MS Fragmentation of HTHLFALR

MS/MS Fragmentation of YNAELESGNQFVLYR
Spot 37

Match to: IPI00210644 Score: 179 Expect: 5e-014

Tax_Id=10116 Gene_Symbol=Cps1 Carbamoyl-phosphate synthase [ammonia], mitochondrial precursor

Nominal mass (M_r): 164476; Calculated pI value: 6.33

Sequence Coverage: 22%

Matched peptides shown in Bold Red

1 MTRILTACKV VKTLKSGFGL ANVTSKRQWD FSRPCGIRLLS VKAQTAHIVL
51 EDGTMKGYS FGHPSSVAGE VFVNTGLGGY SEALTDPAYK GQILMTANPI
101 IGNGGAPDTT ARDELGLNKY MESDGIKVAG LLVLNYSHDY NHWLATKSLG
151 QWQLQEEKVFA IYGVDRTRMLT KIIRDGTLTGK IGEFEQGSV DFVDPNQKQL
201 IAEEVTDKDVK VFGKGNFTKV YAVDCAIQKKN VIRLVLKV RGA EVHLVWPWHD
251 FTQMDXDLGL IAGGPGNPAL AQPLIQNKV VKLGVREKPL FGISTGNIIT
301 GLAAGAKEYK MSNARNQGQNG PVLNITNRQA FITAQNHGAY LLDNLFAGWK
351 PLFVNVNDQT NEGIMIESKP FFARQFHIPEV SPGPIITDEYL FDSKFSLIKK
401 GKGTTITSLVP PKPALVAYS VEVSKVILGSS GLSLIQGAGE FDYSQGAVK
451 AMKEENVKTV LMNPJASQVL TNEVGLKQAD AYFPLPTTPQ FYTVEKAER
501 PDGLILGMGG QTALNCNGVEL FKRGLVKEYG VKLGLTSVES IMATEDQFL
551 SDKLNEINEK IAPSFAVEM KSADLAADTI GYPVMIRAY ALGGLGSGIC
601 PKXETMLDGL TKAFAKTMVILQ LVERSVTGK EIEEYEVVDRA DDNCVTVCNM
651 ENVDEMGMVH GDSSVVAAPAQL TSLNSAEPQML RRTSINVRHR LGIVGECNIQ
701 FALHPTSMVEY C1IEVNRALS RSSALASKAT GYPLAFIAAK IALGIPLPEI
751 KNVNSGTKSA CTEPSLGYVMK TIKPWRWDLR FGHTSSRIGS SMKSVGEPMA
801 IGRTFEESFQ KALRMCPHSVP DGFTPLPMN KEWPANLDRK KELSEPSSTR
851 IYAIKALEN NMSLDEIVKL TSIKDIWLKY MRDILDMDKTK LGLLNSESVT
901 EETLRAKKEI GFSDKQRSKKG LGLTEAQTR ERLKKNIHPW VKDILDCLA
951 YPSVTNYLV TYNQOEHDIK FDEHGIMVGL CGPQHGGVSV EFDWCAVSSI
1001 RTLRLQGKXT VVVCNPEVT STDFDCKDL YFEELSLERI LDIYHQEEACN
1051 GC1ISVGGQI PNLLAVLYK NGVKIMCTSP LQIDRAEDRS IPSAVDSLEK
1101 VAQAPKAVXN TLNEALEFAN SVGYCPCLLRP SYVLGSSAMN VFSEDEMRK
1151 FLEETAVSQ EHPVLTKFI EGEREMEDA VGKEGRVISH AISHEVEDAG
1201 VHSGDATLML PTQTISQGAI EKVKDAKRK IAFAIYGSPF NVQFLVNGDX
1251 VLYIECNRKA SRSFFPSKST LGVDFIVDAT KVMSVESVDE KHLPTLEQP1
1301 IPDYSVAIKF PMFSWPRMLRD ADIPLRCEMA STGEVACFGE GIHTAFLKAM
1351 LSTGFKIPQK GILIGIQQSF RPRFLGVAGQ LHNEFGLK LFA TEATSDWLANA
1401 NNPATPVAV PSQEGQPNPL SSIRKLRDG SIDLVLINPN NNTKVFHNDY
1451 VIRRTAVSDG 1ALLTNFQVT KLFAEAVQKA RTVDSSKSLFH YRQYSAGKAA
1501

Probability Based Mowse Score

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.

Protein scores greater than 59 are significant (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

MS/MS Fragmentation of $\text{MCHPSVDGFTP}$

MS/MS Fragmentation of $\text{SLFHYR}$

MS/MS Fragmentation of $\text{GILIGIQQSFRPR}$

MS/MS Fragmentation of $\text{GQNQPVLNITNR}$
Spot 1743
Match to: IPI00231963 Score: 376 Expect: 1e-033
Tax_Id=10116 Gene_Symbol=Ddt D-dopachrome decarboxylase
Nominal mass (M): 13125; Calculated pI value: 6.09
Sequence Coverage: 79%
Matched peptides shown in Bold Red

1 MPVFELETNL PASRIPAGLE NRLCAATATI LDKPEDRVSV TIRPGWTLLM
51 NKSTEPCAHLI ISSIGVGT AEQNRS HHSSS FFKLTEEELS LDQDRIIRFL
101 FPLEPWIQIK KGTVMFL

Probability Based Mowse Score
Ions score is \(-10\log(P)\), where P is the probability that the observed match is a random event.
Protein scores greater than 59 are significant (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

MS/MS Fragmentation of FLTEELSLDQDR

MS/MS Fragmentation of STEPCAHLI SSIGVGTAEQNR
MS/MS Fragmentation of VSVTIRPGMTLLMNK

MS/MS Fragmentation of LCAATATILDKPEDR

MS/MS Fragmentation of IPAGLENR
**Spot 1708**

Match to: IPI00324380  Score: 146  Expect: 1e-010

**Tax_Id=10116 Gene_Symbol=Ttr Transthyretin precursor**

Nominal mass ($M_r$): 15710; Calculated pI value: 5.77

Sequence Coverage: 20%

Matched peptides shown in **Bold Red**

1  MASLRLFLLC LAGLIFASEA GPGGAGESKC PLMVKLDVAV RGSPADVAV

51  KVFKKTADGS WEPFASKTA ESGLHGLTT DEK PTEGYR VELDTKSYWK

101  ALGISPFHEY AEVVFTANDS GHRHYTIAAL LSPYSYTTA VVSNPQN

**Probability Based Mowse Score**

Ions score is $-10\times\log(P)$, where $P$ is the probability that the observed match is a random event.

Protein scores greater than 59 are significant ($p<0.05$).

Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

---

**MS/MS Fragmentation of ALGISPFHEYAEVVFTANDSGHR**
Spot 1168

Match to: IPI00327518 Score: 334 Expect: 1.6e-029

Tax_Id=10116 Gene_Symbol=Arg1 Arginase-1

Nominal mass (M_r): 36481; Calculated pI value: 6.51

Sequence Coverage: 62%

Matched peptides shown in **Bold Red**

1 MQQQQQQEPW MSMSKPKPI E1GAPFSKG QPRGGVEKGP AALRKAGLVE

51 KLKTEYNVR DHSGLAFVDV PNDDPFQIVK NPRSVMKANE QLAAVVAEQ

101 KNHTISVLG GDHSMAIGSI SGHARVHPDL CVIWVDAHDT INTPLTSSG

151 NLHGQPVAFL LKELKGFPD VPGFSWVTCP ISAQDIVYIG LRDVDPGEHY

201 IITLGLKYP SMTEVDSLGI GKVMEETF SY LLGRKKRPIH LSFVDGDLDP

251 VFTPATGTPV VGGLSYREGL YITEEYKTG LLSDLIDMEV NPTLGTKPEE

301 VTRTVNTAVA LTLSCFGTKE EGNHKPETDY LKPPK

**Probability Based Mowse Score**

Ions score is $-10 \cdot \log(P)$, where $P$ is the probability that the observed match is a random event.

Protein scores greater than 59 are significant ($p<0.05$).

Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

---

**MS/MS Fragmentation of VMEETFSYLLGR**

---

**MS/MS Fragmentation of VMEETFSYLLGR**
MS/MS Fragmentation of **DIVYIGLR**

MS/MS Fragmentation of **DHGDLAFVDVPNDSPFQIVK**
Spot 564
Match to: IPI00475676 Score: 125 Expect: 1.3e-008
Tax_Id=10116 Gene_Symbol= Tas1r2 Delta-1-pyrroline-5-carboxylate dehydrogenase, mitochondrial precurs
Nominal mass ($M_r$): 61830; Calculated pI value: 7.14
Sequence Coverage: 26%
Matched peptides shown in **Bold Red**

1 MLPPALLRRS LLSYAWRGG SGLRWKHASSLK **VANEPIALT QGSPERDALQ**
51 KALNDLKDQT EAIPCVGDGE EVWTSDVRQ **LSPFNHGKV AKFCYADVJ**
101 LNK**AIEAAVL ARKEWDLKPV ADRAQIYLA** ADMLSGPRRA EILAKTMGQ
151 GKT**VIQAEID AAAELIDFRR FNAKFAEVE GEQIPSVPRS TNIHYVGRLE**
201 GFVAAISPEN FTA1GGNLG APALMGNVVL WKPSDTAMLAR SYAVYRILRE
251 AGLPPNVIQF VPADGPTFQD TVTTSEHLCG INFTGSVPFT KHLWKQVQNY
301 LDRFRFTFPRL AGECCGK**NFH FVHSSHADVDS VVSGLRLSAP EYGGQKCSAC**
351 SRLYVPQSLW P1QNGRLLIE HSR1KVGNP AEDFQTFTSVAV IDAKAFARIK
401 K**WLEHARSSP SLSILAGGQC NESVGYFVEP C1IESKDPQG P1MKKEIFGP**
451 VLT**TVYYPDE KYRETLQLV S**TTSYLGTA VFAQDKT**IVQ EATRMLT**
501 GNFY**NDKST GS**VVGQPQFGGAR ASGERDI PGQPRLVL**QW TEPPFTPLAV
551 S**PPLGWDRYR**S YMQ

**Probability Based Mowse Score**

Ions score is $-10*\log(P)$, where $P$ is the probability that the observed match is a random event.

Protein scores greater than 59 are significant ($p<0.05$).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

![Probability Based Mowse Score](image)

**MS/MS Fragmentation of STGSVVGQPQFGGAR**
Spot 1400

Match to: IPI00365297 Score: 290 Expect: 4e-025

Tax_Id=10116 Gene_Symbol=Agmat Agmatinase, mitochondrial precursor
Nominal mass (M_r): 37963; Calculated pI value: 6.71
Sequence Coverage: 32%
Matched peptides shown in Bold Red

1  MLQLLKSSWV RSAGSVVTW RASAGLFCGP TRQASDTSDT LHHPSPSSES
   51  QVQPVRYCSM MHPLQQSPE GLDAFVGP LDGTGSNRPG ARFGPRKIRE
   101  ESLMLGTYNS MGHALPNVNL NLYNLQDSLE LIREAYQNIL
   151  ATGCIPITLG GDHITTYPL QAVAKEHGVP GLVHVGAHSN TSDKPLEDQ
   201  YHRTPFRRSV DEGLDSKRV VQIGIRGSSR TLDPRYRYSRS QGFRVVLAD
   251  CWMKSLVPLM AEIRQQMGGV PLYISFAIDA LDPAYAPGTG TPEIAGLTPS
   301  QALEIIRGCQ GLNVGCALDV EVSPPYDLG NTALLAANLL FEMLCALPKV
   351  TTV

Probability Based Mowse Score

Ions score is $-10\log(P)$, where $P$ is the probability that the observed match is a random event.

Protein scores greater than 59 are significant ($p<0.05$).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.
MS/MS Fragmentation of TLDPYR

MS/MS Fragmentation of SLVPLMAEIR

MS/MS Fragmentation of VVQIGIR
MS/MS Fragmentation of VADLGNVNVNLYNLQDSCR

Spot 560
Match to: IPI00231742 Score: 71 Expect: 0.0031
Tax_Id=10116 Gene_Symbol=Cat Catalase
Nominal mass (M_r): 59719; Calculated pI value: 7.07
Sequence Coverage: 22%
Matched peptides shown in Bold Red

Probability Based Mowse Score

Ions score is $-10 \log(P)$, where P is the probability that the observed match is a random event.
Protein scores greater than 59 are significant \( (p<0.05) \).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

MS/MS Fragmentation of LGPNYLQIPVNCPYR

**Spot 527**

Match to: IPI00327469 Score: 101 Expect: 3.2e-006

**Tax_Id=10116 Gene Symbol=Ahsg Alpha-2-HS-glycoprotein precursor**

Nominal mass \( (M_r) \): 37958; Calculated pI value: 6.05

Sequence Coverage: 21%

Matched peptides shown in **Bold Red**

| Peptides | Score |
|----------|-------|
| MKSLVLLLCF AQLWSCQSAP QGAGLGFRQIL ACDDPETEHV ALI AVDLNLK | 1 |
| HLLQGRQIL NQIDKVKWS RRPFGEYEL EIDLLETCH ALDPTPLANC | 51 |
| SVQQAEHAY GDCDHFILK QDGQRVLALHA QCSTPDSEAE DVRKFPYRCP | 101 |
| ILIRFNDTNV VHTVKHALAA FNAQQNGTYF KLVEISRAQ YVFPYSTLYE | 151 |
| FVIAATDCGQ EVTDPAKCN LLAEKQYFC KATLIHR LGG EEVS VACKLF | 201 |
| QTQPQPANAN PAGPACTVGQ AAPVAPPAPS PESVYVGPA VPLGLPDHRT | 251 |
| HHDLRHAFSP VASEVASEG VLHSPKQGQP GDAGAAGPVA PPLCPWVRYF | 301 |
| 351 | K |

**Probability Based Mowse Score**

Ions score is \(-10\log(P)\), where \( P \) is the probability that the observed match is a random event.

Protein scores greater than 59 are significant \( (p<0.05) \).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.
Spot 543

Match to: IPI00206624 Score: 206 Expect: 1e-016
Tax_Id=10116 Gene_Symbol=Hspa5 78 kDa glucose-regulated protein precursor
Nominal mass (M_r): 72302; Calculated pI value: 5.07
Sequence Coverage: 41%
Matched peptides shown in Bold Red

1 MKFTVAAAL LLLCAVRAEE EDKKEDVGTG VGIDLGTTYS CVGVFKNGR
51 EIIANDQCN ITPSYVAFTP EGERLIGDAA KNQLTSNPEN TVFDARKLIG
101 RTWNDPSVQQ DIKFLPKVY EKKTKPYIQV DIGGQRTKTF APEEISAMVL
151 TKMKEAAY LGKKVTHAVV TVPAYFNDAL AQQTDDAGTI AGLNWMIIIN
201 EPTAAAIAYG LDKREGEKNI LVFDLGGGTDF VSLLITIDNG VFEVVATNGD
251 THLGGEDFDQ RVMIEFIKLY KKKTKGDRVK DNRAVQKLRR EVERAKRALS
301 SQHQRARIEIE SFFEDEDSEE TLTRKFEEL NMDFRSTMK PVQVLEDSD
351 LKKSDIDEIV LGGSTRIPK IQQLVKEFFN GKEPSRGINP DEAVAYGAAV
401 QAGVLSGDQD TGDLVLLDVC PLTLGIETVG GVMTKLIPRN TVVPTKSKQI
451 FSTASDNQPT VTIKYVEGER PLTKDNHLLG TPFDTGIPPA PRGVPQIEVT
501 FEIDVNGILR VTAEDKGTGN KKKITTNDQ NKLTPEEIER MVHDAEKFAE
551 EDKILKIRID TRNELESYAY SLKNQIGDKE KLGKLSPED KETMEKAVEE
601 KIEWLESHQD ADIEDFKK AK KELEEIVQPI ISKLYGSGGP PPTGEEDTSE
651 KDEL
Probability Based Mowse Score

Ions score is $-10 \times \log(P)$, where $P$ is the probability that the observed match is a random event.
Protein scores greater than 59 are significant ($p<0.05$).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

MS/MS Fragmentation of DNHLLGTFDLTGIPPAPR

MS/MS Fragmentation of VTHAVVTVPAYFNDQAQR

MS/MS Fragmentation of ITPSYVAFTPEGER
Spot 1514
Match to: IPI00230788 Score: 468 Expect: 6.4e-043
Tax_Id=10116 Gene_Symbol=Ca3 Carbonic anhydrase 3
Nominal mass (M): 29413; Calculated pI value: 6.89
Sequence Coverage: 85%
Matched peptides shown in Bold Red

1  MAKWGYASHNGPEHWHELP1AKDNQSPIELHTKD1RHDPSSLQPWSVS
51  YDPGSALTILNGKTCRVVFDDDTFDRSMLRGGPLSGPYRRLQHILWGSS
101  DDHGSEHTVDDGKYAALHLVHWNPKNTFGEALKQPDGIAVGIFLKG
151  REKGEQYLILLALDRKIKTKGKEAPPNHFDPSCLPACRDFDYWTYHGSFTTP
201  PCCECIVWLLKEPMTTYSDQMAKLRSLSAENEPPVPLVGNWRPPQPI
251  KGRVVRASFK

Probability Based Mowse Score

Ions score is $-10 \times \log(P)$, where $P$ is the probability that the observed match is a random event.
Protein scores greater than 59 are significant ($p<0.05$).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

MS/MS Fragmentation of SLFASAENEPPVPLVGNWRPPQPIK
Spot 799
Match to: IPI00389571 Score: 465 Expect: 1.3e-042
Tax_Id=10116 Gene_Symbol=Krt2-8 Keratin, type II cytoskeletal 8
Nominal mass (M_r): 53985; Calculated pI value: 5.83
Sequence Coverage: 55%
Matched peptides shown in Bold Red

1 MSVRVTQKS YKMSTSGPRAF SSRSFSGPG ARISSSSFSR VGSSSSSF RG
51 SLGFGGAGV GGTAVTVNQ SSLNLKLEVE DPNIQAVRTQ EKEQIKTLNN
101 KFASFIDKVR FLEQQNKMLE TKWSLLQQQK TSRSNMDNMF ESYINNLRQ
151 LEALGQEKLL LEVELGNAQG LVEDFKNAYE DEINKRTEME NEFVLIKKDV
201 DEAYMNKVEL ESRLEGLDTE INFLQIHEE EIRELSQGIS DTSVILMDN
251 SRSLDMDSII AEVRAGYEET ANRSRUEAET MYQIKYEELQ TLAGKHDGL
301 RRSKTEISEM NRNSRLQAE IDALKGQRT LEAAIADAEQ RGELOVKDAN
351 AKLEDLQANL QKAKQDMARQ LREYQELMNV KLALDIEIAT YRKILEEES
401 RLESQMQNMS HHTITSGYGA GGLSSSYGGL TSPGFSGYSMS SFQPGFSGVG
451 GSSTYSRTKA VVVKIETRD GKLVSEESSDI MSK

Probability Based Mowse Score

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 59 are significant (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.
MS/MS Fragmentation of **LALDIEIATYR**

MS/MS Fragmentation of **LQAEIDALKGQR**

MS/MS Fragmentation of **LEGLTDEINFLR**
Spot 1662
Match to: IPI00561017 Score: 488 Expect: 6.4e-045
Tax_Id=10116 Gene_Symbol=Prdx5 22 kDa protein
Nominal mass (M_r): 22255; Calculated pI value: 8.54
Sequence Coverage: 55%
Matched peptides shown in Bold Red

1 MVQLRFCVLG SIAGSVLRA S ATWTCVAGRA GRKGAGWECG GARSFSSRGD
51 YGPDPQGVGDT IPSVEVFEGE PGK KVNLAEL FKD KK GVLFG VPGAFTPGCS
101 KTHLPFGEQ AGALKAKGAQ VVACLSVNDV FVTAEVGRAH QAEKVQLLA
151 DPTGAFKGET DLLLDSLVS LPGNRLKRF SMVIDKGVVK ALNVEPDTG
201 LTCSLAPNIL SQL

Probability Based Mowse Score

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 59 are significant (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

MS/MS Fragmentation of ETDLLLDDSLVSLFGNR

MS/MS Fragmentation of GAQVACL Sv NDVF VTA EWGR
MS/MS Fragmentation of THLPGFVEQAGALK

MS/MS Fragmentation of GVLFGVPAGAFTPGCSK
**Spot 1526**

Match to: IPI00760117 Score: 143 Expect: 2e-010

Tax_Id=10116 Gene_Symbol=Comt Isoform 2 of Catechol O-methyltransferase

Nominal mass (M_r): 24731; Calculated pI value: 5.11

Sequence Coverage: 55%

Matched peptides shown in **Bold Red**

1 MGDTKEQRIL RYVQQNAKPG DPQSVLEAID TYCTQKEWAM NVGDARKQIM
51 DAVIREYSPSYLLELGAAYCGYSVARMLLQPGARLLTEMNPDYAAITQ
101 QMLNFAGLQDKVTILNGASQDLIPQLKKYDVDTLMVFLDHWDRYLPD
151 TLLLEKCGDLLRGKTVLLADNVIVPGTPDFLAYVRGSSSFECTHYSSLEY
201 MKVVDGLEKAIYQGPSPDKS

**Probability Based Mowse Score**

Ions score is $-10 \times \log(P)$, where $P$ is the probability that the observed match is a random event.

Protein scores greater than 59 are significant ($p<0.05$).

Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

![Graph showing probability based Mowse score](image)

**MS/MS Fragmentation of LLQPGAR**

![MS/MS Fragmentation](image)

**MS/MS Fragmentation of EYSPSLVLELGAYCGYSAVR**

![MS/MS Fragmentation](image)
Spot 1563
Match to: IPI00392676 Score: 233 Expect: 2e-019
Tax_Id=10116 Gene_Symbol=Blvrb_predicted biliverdin reductase B
Nominal mass (M_r): 22083; Calculated pI value: 6.29
Sequence Coverage: 62%
Matched peptides shown in Bold Red

1 MAVKKIAIFG ATGRTLTL AQASPSYEV TVLVRDSSRL PSEGPAHV
51 VVDVLQAGD VKTVAGQQA VIVLGTGND LSPTTMSEG TRNIVAAMKA
101 HGVDKVACT SAFLWDPFSK VPPRLQVTD DHIWttvK TLE ESLKYVAVM
151 PPHIGDQPLT GAYTVTLGDR GFSRVISKHD LCHFMLRCLT THEYDQKTY
201 PSHQVD

Probability Based Mowse Score

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 59 are significant (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

MS/MS Fragmentation of HDLGHFMLR

MS/MS Fragmentation of YVAVMPPHIGDQPLTGAYTVTLGDR
MS/MS Fragmentation of IAIFGATGR
**Spot 1746**

Match to: IPI00205036 Score: 534 Expect: 1.6e-049  
Tax_Id=10116 Gene_Symbol=LOC360504 hemoglobin alpha 2 chain  
Nominal mass (M): 15275; Calculated pI value: 8.45  
Sequence Coverage: 88%  
Matched peptides shown in **Bold Red**

1. MVLSAADKTN IKNCWGIIGG HGGEYGEEAL QRMFAAFPTT KTYFSHIDVS  
51. PGSAQVKAHG KKVADALAKA ADHVEDLPGA LSTLSLHAAH KLRVDPVFNK  
101. FLSHCLLVTL ACHHPGDFTP AMHASLDKFL ASVSTVLTSDK YR

**Probability Based Mowse Score**

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.  
Protein scores greater than 59 are significant (p<0.05).  
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

---

**MS/MS Fragmentation of IGGHGGEYGEEALQR**

---

**MS/MS Fragmentation of TYFSHIDVSPGSAQVK**
MS/MS Fragmentation of AADHVEDLPALSTLSLHAK

MS/MS Fragmentation of LRVDPVNFK
Spot 1453
Match to: IPI00231106 Score: 405 Expect: 1.3e-036
Tax_Id=10116 Gene_Symbol=Mpst 3-mercaptopyruvate sulfurtransferase
Nominal mass (M_r): 32919; Calculated pI value: 5.88
Sequence Coverage: 57%
Matched peptides shown in Bold Red
1 MAAPQLFRAL VSAQVAEAEL KSQRASQPFLK LLDASWYLPK LGRDARREFE
51 ERHIPGAFF DIDRCSDHTS PYDHMLPSAT HFADYAGSLG VSAATHVVIY
101 DGSDQGILSYAPRVVWMRAF GHSVSLLDG GFRIWLQNL PISSGKSPSE
151 PAEFCQAQLDP SFIKTEDIL ENLDARRFQV VDARAAGRFQ GTQPEPRDG
201 EPCHIPGSYIN IPFTEFLTSE GLEKSPEEQ RLHQQKVLG SKPLVATCGS
251 GVTACHVVLG AFLCGKPDPVP VYDGSWVEWY MRAQPEHVIS QGRGKTL

Probability Based Mowse Score

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 59 are significant (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

MS/MS Fragmentation of AQPEHVISQGR

MS/MS Fragmentation of FQGTQPEPR
MS/MS Fragmentation of AFGHHSVLLDGGFR

MS/MS Fragmentation of HIPGAAFFDIDR