

RESEARCH ARTICLE

The different expressed serum proteins in rhCygb treated rat model of liver fibrosis by the optimized two-dimensional gel electrophoresis

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Abstract

Liver fibrosis, a common pathological process of chronic liver diseases, is the final stage of liver dysfunction that has severely deleterious impact on human health. Cytochrome b was first discovered in 2001 by proteomic analysis in rat stellate cells and was reported to play an important role in controlling tissue fibrosis. However, the mechanism by which cytochrome b inhibits or reverses the progression of fibrosis remains unclear. The present study examines the effect of recombinant human cytochrome b (rhCygb) in a rat model of liver fibrosis. Proteomic approaches were employed to identify differentially expressed proteins in the fibrosis model. Optimized conditions for two-dimensional gel electrophoresis were developed to provide improved protein detection and separation. A total of 43 spots were obtained and, through the use of matrix-assisted laser desorption ionization time-of-flight mass spectrometry, 30 differentially expressed proteins were identified. Gene ontology term annotation and KEGG pathway analysis allowed us to explore the function of the represented proteins. Based on these results, we provide a theory of the molecular mechanism related to rhCygb reversion of fibrosis and which will assist in the identification of biomarkers in patient serum to improve early diagnosis of liver fibrosis.

Introduction

Liver fibrosis is a response to chronic liver injury and is caused by a series of actors[1, 2]. It is well-established that liver fibrosis is a total pathological change induced by sustained liver injury, liver cell necrosis or inflammation. The underlying pathological process involves the activation of quiescent hepatic stellate cells (HSCs) into contractile myofibroblast-like cells, which secrete excessive extracellular matrix (ECM) proteins, including collagen, in the liver [3]. The inflammation resulting from liver fibrosis is difficult to repair and may ultimately lead to liver disease or cancer. Therefore, any reversal of advanced liver fibrosis prior to cirrhosis would be of great importance to human health[4].

Cytoglobin (Cygb) is a hexacoordinated heme-containing globin, identified in recent years [5], located in the fibroblast cytoplasm and nucleus of most visceral tissue. As a member of the globin family [6], which includes myoglobin, hemoglobin, and neuroglobin, Cygb has the same function of binding and transporting oxygen. In particular, Cygb is expressed ubiquitously in the cytoplasm of pericytes in many organs, including the brain, thymus, heart, lung, liver, kidney, small intestine and spleen [7]. One interesting aspect of Cygb expression is its presence in visceral cells that have the ability to store vitamin A. It has been reported that Cygb protects cells from reactive oxygen with its functions of O₂ storage, diffusion and sensing for cellular respiration and metabolism, NO scavenging and involvement in hypoxia and oxidative stress [8–10]. These findings prompted the hypothesis that Cygb could be effective in the prevention or treatment of liver fibrosis [11]. The molecular mechanism for this hypothesis is still not clear, but its nitrogen oxide dioxygenase activity and lipid peroxidase activity have been demonstrated [10].

Recently, proteomic approaches have been utilized to identify protein markers for specific diseases or disease stages [12]. Liver biopsy remains the gold standard by which to assess hepatic fibrosis, but it is invasive, expensive and often poorly tolerated by patients [13]. For these reasons, it is desirable to predict hepatic fibrosis without invasive liver biopsy. An easily obtained sample of serum or plasma is the promising choice for biomarker studies [14]. Proteomic techniques allow unbiased assessment of proteins and may be useful to identify proteins related to hepatic fibrosis. Two-dimensional gel electrophoresis (2-DE) is a common approach for proteomic analysis which separates and identifies hundreds of proteins [15–17]. However the proteomic analysis of serum samples remains a challenge due to the presence of a few highly abundant proteins, such as albumin and immunoglobulins. Removal of uninformative, high expression proteins is desired to enhance the sensitivity of analysis to the disease related protein of interest [18–20]. In this study, rat serum was pretreated with a 2DE Clean-Up kit to remove interfering components, such as high concentrations of salt ions, lipids, and polysaccharides. The samples were further refined using a ProteoMiner kit, employing a large bead-based library of combinatorial peptide ligands, to dilute high-abundance proteins and concentrate low-abundance proteins, or an Albumin/IgG removal kit. A comparison of the effectiveness of these protein isolation and enhancement technologies allowed us to optimize pretreatment conditions for 2-DE. Finally, the serum samples were analyzed by 2-DE to identify a protein panel of possible biomarkers in the liver fibrosis model.

Materials and methods

Reagents

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were purchased from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). Hyaluronic acid (HA), laminin (LN), collagen III (Col III) and collagen IV (Col IV) ELISA kits were obtained from Abbott Laboratories, USA. A Bradford kit was bought from Sangon Biotech, Shanghai, China. Sirius red and hematoxylin-eosin (HE), protease inhibitor cocktail, 3-[(3-Cholamidopropyl)dimethylammonio]-propanesulfonate (CHAPS), glycine, ammonium persulfate (APS), TEMED, and protease inhibitor cocktail were obtained from Sigma Chemical (St. Louis, MO, USA). Dithiothreitol (DTT), glycine, Immobilized pH gradient (IPG) strips, IPG buffer, dry strip cover fluid and other reagents that were used in two-dimensional gel electrophoresis were acquired from Bio-Rad (Hercules, CA, USA).

Animals and treatments

Sexually mature Sprague-Dawley (SD) rats (n = 90), weighing 180–220 g, were obtained from the Experimental Animal Center of Southern Medical University, Guangzhou, China.

Approval of Southern Medical University Animal Care and Use Committee (IACUC) Number is 2014–025 and Permit Number is SCXK20110015. Care of the animals used in this investigation was conducted according to the guidelines approved by the Chinese Association of Laboratory Animal Care. Rats were anesthetized with intraperitoneal injections of 2% sodium pentobarbital (50mg/kg body weight) and sacrificed with the theoretical injection of 1.2 to 1.5 times dose according to Southern Medical University IACUC-approved procedures. They were housed in a room under a constant temperature ($20\pm 2^{\circ}\text{C}$) and humidity (70%) with a 12 h light/dark cycle. The rats had free access to standard diet and water. The animals were randomly assigned to two groups, the control group ($n = 30$) and the carbon tetrachloride (CCl_4) induced hepatic injury group ($n = 60$). A rat model of liver fibrosis was created using two treatment groups: a mild model group and medium model group. The mild model group was treated twice per week for 10 weeks with intraperitoneal injections of 25% CCl_4 in paraffin oil at 2 ml/kg. The rats in the moderate liver fibrosis model group were treated as in the mild model group, but received additional treatment for 3 weeks of intraperitoneal injections of 50% CCl_4 twice per week, adjusting the dose for the weight of each rat. Serum levels of ALT, AST, HA, Col III, and Col IV were measured in the two groups. Staining with Sirius red and HE confirmed that CCl_4 treatment successfully induced hepatic fibrosis.

After the fibrosis model was established, liver fibrosis model rats ($n = 30$) were treated daily with rhCygb (2 mg/kg body weight/day) and twice per week with 25% CCl_4 . The production and purification of rhCygb were prepared by our laboratory[11]. Rats were anesthetized and sacrificed within 24 h after the last treatment. Histological examination of the liver and serum parameters were evaluated as before. Blood samples were collected from the abdominal aorta. The serum was separated via centrifugation at 5000rpm for 10 minutes at 4°C and stored at -80°C for further study.

Optimizing methods for 2-DE

Serum samples were obtained from the control group, CCl_4 model group and rhCygb treated group. We explored different kits for pre-treatment and different ranges of IPG strips for 2-DE. The interfering components (high concentrations of salt ions, lipids, and polysaccharides) in the serum supernatant were removed using a 2-D Clean-Up Kit. Further refining of the samples was done with the ProteoMiner kit and compared against samples treated with an Albumin/IgG removal kit in order to provide a valuable tool for enhancing the detection of low-abundance proteins. Briefly, a column from the ProteoMiner Protein Enrichment Kit was loaded with 1ml of serum sample containing a protease inhibitor cocktail and incubated for two hours at room temperature under constant rotation. After incubation, the column was washed 3 times with 500 μL of PBS to remove the unbound high-abundance proteins. The desired proteins were eluted from the column with a 5% acetic acid buffer (3 times) and collected. Also the Albumin/IgG removal kit was carried out according to the manufacturer's instructions. Protein was measured with a Bradford kit. A total of 120 μg of protein in 300 μL 2D sample rehydration buffer was separately loaded onto a 17cm linear IPG strip (pH 5–8) and 17cm nonlinear IPG strip (pH 3–10) for first-dimension isoelectric focusing (IEF). The strips were placed into a Protean IEF cell and rehydrated at 50 volts for 16 hours. IEF was carried out at 250 volts for 1 hour, 500 volts with rapid climbing for 1 hour, 1000 volts with rapid climbing for 1 hour and a final linear rapid voltage gradient to 10,000 volts until reaching 65000 volt-hour (current limit was set at 50 μA at 20°C). Focused strips were held under 500 volts until ready for equilibration. After IEF, the IPG strips were blotted against damp filter paper to remove excess mineral oil and then were equilibrated for 15 minutes in a buffer containing 50mM Tris-HCl, pH8.8, 20% glycerol, 6M urea, 1% sodium dodecyl sulfate (SDS), and

2% DTT. The strips were further treated in a similar buffer (containing 2.5% iodoacetamide) for 15 minutes and washed with SDS electrophoresis buffer before being directly applied into 12.5% homogeneous SDS-PAGE gels. The gels were run in parallel at 10mA for 60 minutes, and then run at 28mA until the bromophenol blue dye reached the bottom. Each experiment was performed in triplicate.

Image analysis and protein identification

Two dimensional gels were stained with the silver method to make it compatible with mass spectrometry. The stained gels were scanned using UMax Magic scan (Amersham Biosciences, Sweden), and analyzed with PDQuest 8.0.1 (Bio-Rad). In-gel digestion and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) for identification of different proteins analysis were performed at the Beijing Genomics Institute in Shenzhen, China. Combined peptide mass fingerprinting (PMF) and tandem mass spectrometry (MS/MS) queries were searched against the NCBI database, using the MASCOT search engine from Matrix Science (<http://www.matrixscience.com/>) to identify the proteins. Proteins matching more than five peptides and with a MASCOT score higher than 60 were considered significant ($p < 0.05$).

Protein interaction analyses and bioinformatics analysis

The web portal for the STRING database (<http://www.string-db.org/>) was used for protein—protein interaction (PPI) network analysis. The networks that involved proteins derived from 2-D DIGE experimentations 30 differentially expressed and identified proteins, and architecture represents connections between the individual proteins. To examine the functions and pathways of the differentially expressed proteins, we searched the UNIPROT database (<http://www.uniprot.org/>) to identify the functions and relevant pathways of these proteins. To functionally annotate the correlated genes, gene ontology (GO) and pathway analysis was conducted using the Database for Annotation, Visualization and Integrated Discovery (DAVID) functional annotation tool (<http://david.ncifcrf.gov/tools.jsp>). Pathway enrichment analysis of the differentially expressed proteins was obtained from the KEGG database.

Statistical analysis

The Statistical Program for Social Sciences (SPSS) version 19 was used for data analysis calculations. All data are expressed as the mean \pm standard error of the mean. The Levene test for homogeneity test of variance and One-way ANOVA was used for comparison among groups. Differences between two groups were compared using an unpaired Student t-test. Values of $p < 0.05$ were considered statistically significant.

Results

CCl₄ induced hepatic fibrosis in rats

After HE and Sirius red staining, livers from the control group showed normal lobular architecture with central venous and hepatic cord radiation (Fig 1A and 1D). The CCl₄ treated group showed extensive disruption of the liver architecture, including hepatocellular necrosis and inflammatory cell infiltration (Fig 1B and 1E). Analysis of serum biochemistry (Table 1) showed that ALT and AST levels were increased ($P < 0.01$) in the fibrosis model group. Serum LN, HA, Col III and Col IV levels in the CCl₄ treated groups were higher than in the control group. These results clearly illustrate that the model of CCl₄-induced fibrosis was successfully established. Meanwhile, the rhCygB treatment group displayed markedly decreased serum

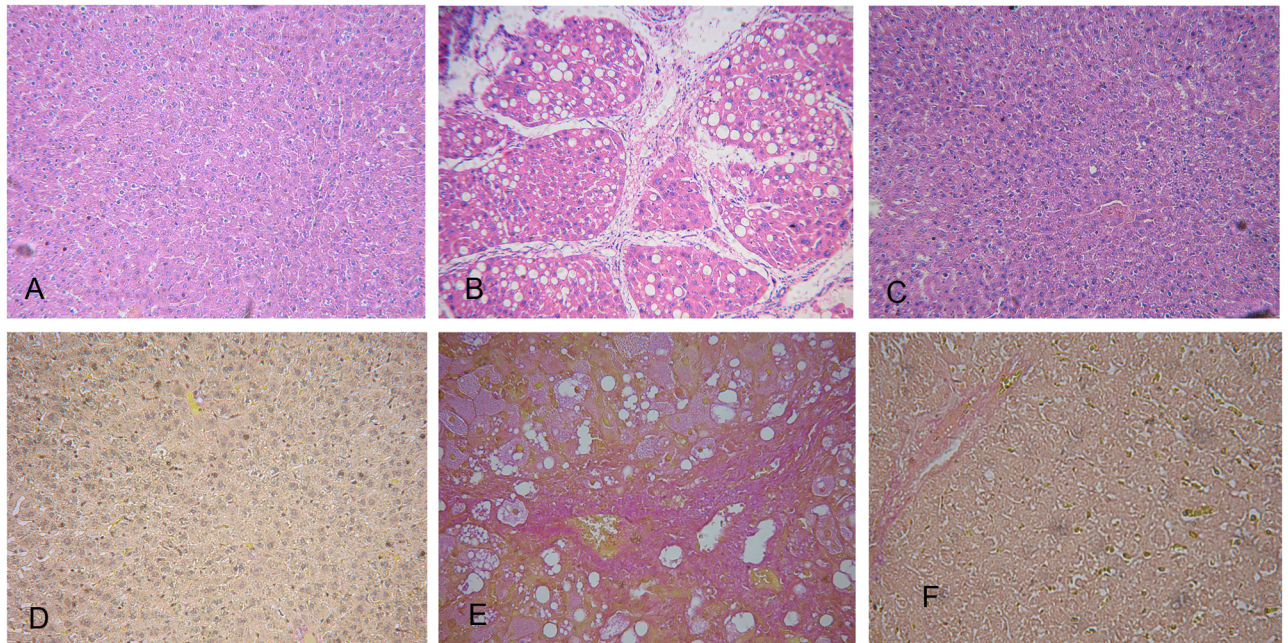


Fig 1. HE and Sirius red staining of liver biopsy samples among the different groups. A, B and C: the HE staining of liver biopsy of the control group, fibrosis model group and rhCygb treatment group, respectively. D, E and F: the Sirius red staining of liver biopsy samples of the control group, fibrosis model group and rhCygb treatment group, respectively.

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levels of these markers, compared with the fibrosis model group. The livers of rats treated with rhCygb displayed lower content of collagen fibers and exhibited a clear improvement when compared with the CCl₄ treated group (Fig 1C and 1F).

2-DE pattern

To optimize conditions for the analysis of rat serum via 2-DE, a 2-D Clean-Up Kit was used, according to manufacturer’s recommendations, to remove interfering components and to determine the proper loading quantity of samples (120µg of protein) and the optimal 17cm nonlinear IPG strip (pH 3–10) for IEF. The ProteoMiner kit treatment showed a greater benefit for enriching low-abundance species, compared with the Albumin/IgG removal kit, as shown in Fig 2. Using 2-DE, proteins from the control, fibrosis model and rhCygb treatment groups are displayed in Fig 3. Each experiment was performed in triplicate and analyzed with PDQuest 8.0.1 software. We obtained 812 ± 28 protein spots with an 88.2 ± 7% matching rate.

Table 1. Anti-hepatic fibrosis effect of rhCygb in CCl₄-induced liver fibrosis model rats($\bar{x} \pm s$, n = 30).

Groups	ALT(U/L)	AST(U/L)	LN(ng/ml)	HA(ng/ml)	Col III(ng/ml)	Col IV(ng/ml)
Control	35.52±4.3	37.58±6.35	26.45±6.35	97.33±15.26	14.3±3.5	26.35±5.23
Model	521.46±98.77a	622.33±98.77a	103.75±12.44a	378.95±48.23a	78.23±14.6a	58.78±7.89a
rhCygb	47.25±7.6c	55.26±7.6c	28.52±7.6c	108.56±25.68c	17.2±3.3c	28.24±5.66c

^a Compared with control group P<0.01;

^c Compared with model group P<0.01

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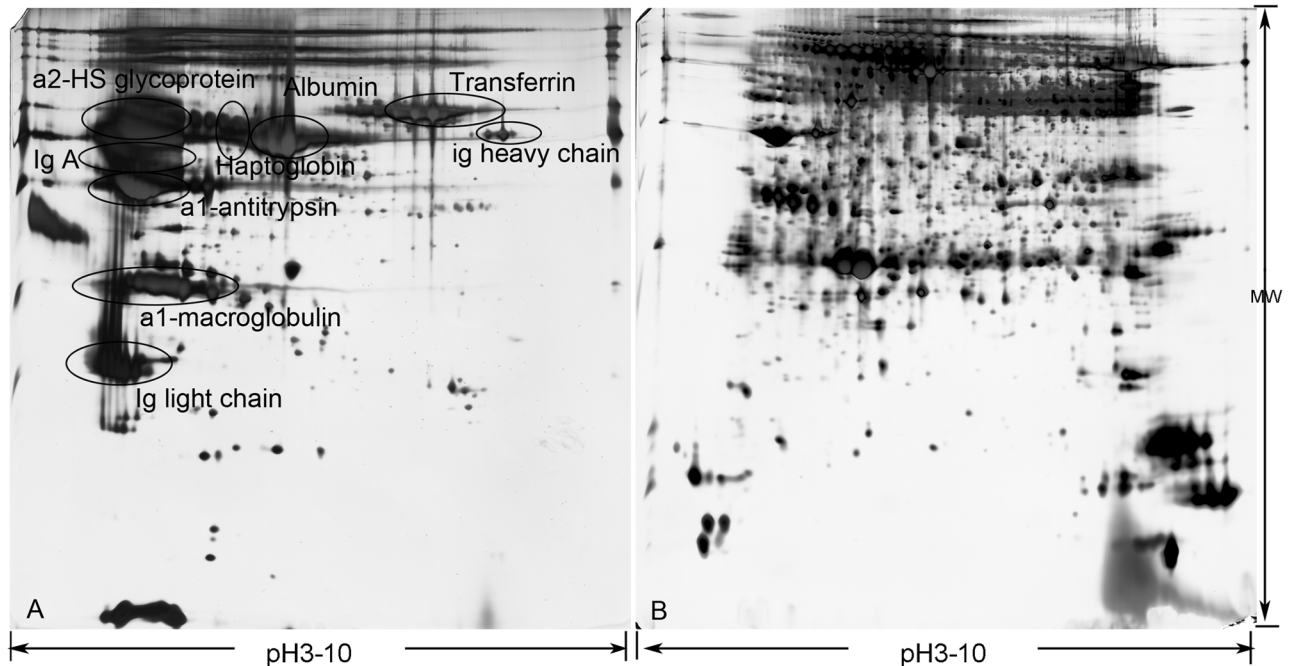


Fig 2. Effect after treatment with different kits on 2-DE of serum proteome. A: Using the Albumin/IgG removal kit, the proteins did not separate well. The main high-abundance proteins were α 2-HS glycoprotein, albumin, transferrin, IgA, haptoglobin, Ig heavy chain, α 1-antitrypsin, α 1-macroglobulin and Ig light chain. B: Use of the ProteinMiner Protein Enrichment Kit resulted in proteins that were separated by a large degree.

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Proteins identified by MALDI-TOF-MS

Represented protein spots were excised from the 2-DE gel and analyzed for protein identification by MALDI-TOF-MS. The PMF was obtained and analyzed using the MASCOT search engine against the NCBI database. The database query resulted in 30 significant proteins and their functions, as shown in [Table 2](#).

Protein—Protein interaction network and GO enrichment pathway analysis

Information on Protein—Protein Interaction was obtained from STRING database ([Fig 4](#)). The networks that involved proteins derived from 2-D DIGE experimentations 30 differentially expressed and identified proteins. To examine the functions and pathways of the 30 up-regulated and down-regulated proteins, the enriched GO terms were categorized for liver fibrosis networks to identify their functional roles. According to the GO categories, the identified proteins could be divided into several relevant metabolic processes ([Fig 5](#)), which included responses to oxidative stress and stimulus, NF- κ B signaling, catabolic processes and antioxidant activity. The differentially expressed proteins, and their functional categories, are summarized in [Table 3](#).

Discussion

In the present study, we used CCl₄ to establish a liver fibrosis model [[2](#), [21](#), [22](#)], as confirmed by histological evaluation and the increased levels of the Serum parameters ALT, AST, HA, LN, Col III and Col IV in the model group, compared with the control group. After treatment with rhCygb, the levels of serum markers were decreased, compared with the fibrosis model

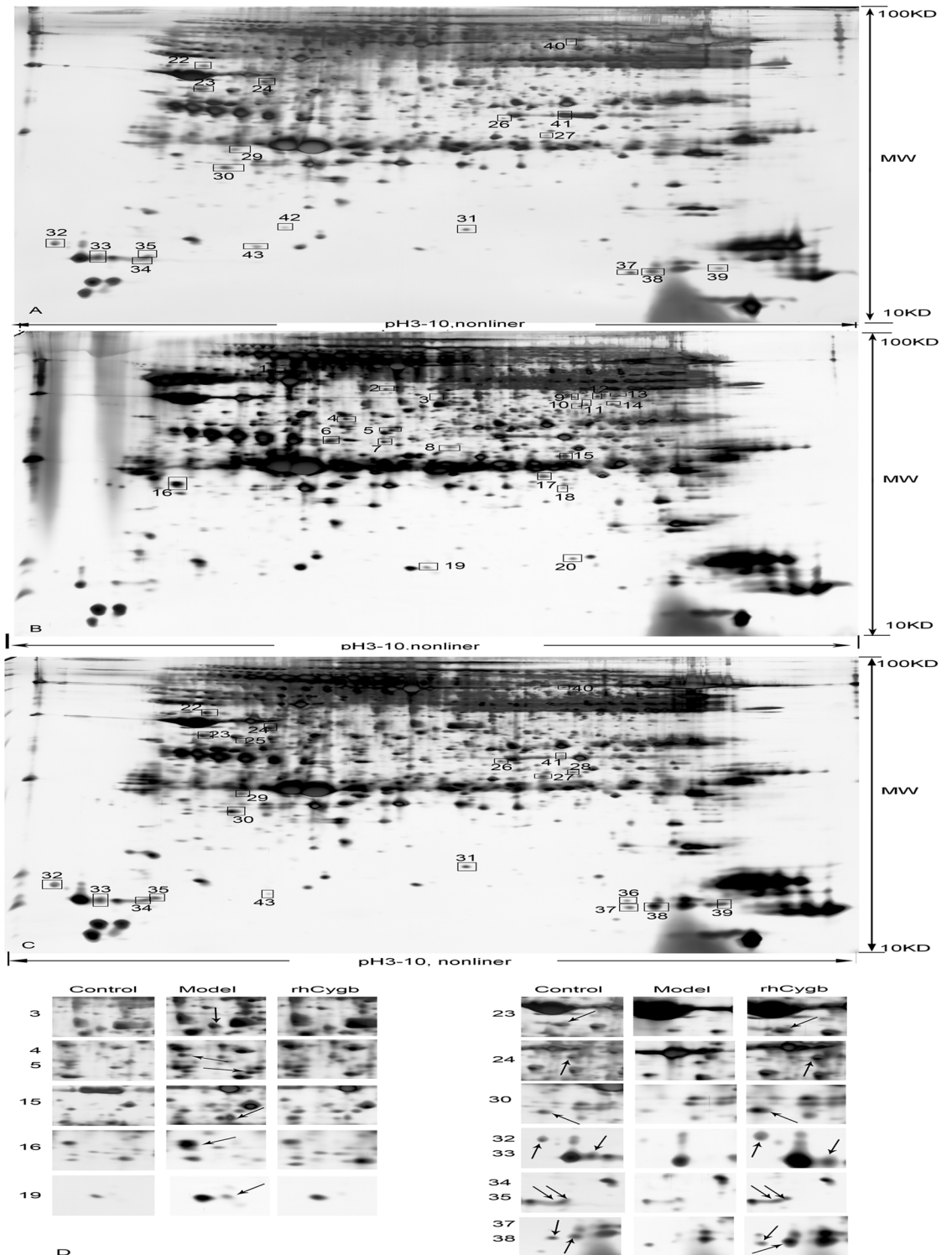


Fig 3. 2-DE analysis of serum samples and the corresponding differentially expressed protein spots. A: Control group, B: Fibrosis model group, C: rhCygb treatment group, D: Fifteen representative differentially expressed protein spots were magnified from three pairs of serum samples. The spots on the left side of the figure were up-regulated in fibrosis model group, while spots on the right were down-regulated in the fibrosis model group. Using a pH3-10, 17cm nonlinear IPG Strip and 12.5%SDS-PAGE, we identified 43 different proteins on the gels.

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Table 2. Identification of 30 proteins differentially expressed among the control, fibrosis model, and rhCygb groups.

ID	Protein name	Swiss-prot Accession	MW	PI	Protein Score	rhCygb	Protein function
1	60 kDa heat shock protein, mitochondrial	P63039	610088.45	5.96	269	↓	Positive regulation of inflammatory response; Response to hypoxia
2	Argininosuccinate lyase	P20673	51643.24	6.20	112	↓	Cellular response to hypoxia; Cellular response to tumor necrosis factor; Diaphragm development; Arginine biosynthetic process
5	FGFR1 oncogene partner 2 homolog	Q6TA25	25048.81	5.27	62.8	↓	Response to wounding; Wound healing; Accelerates the collagen gel contraction in vitro
6	Clusterin	P05371	51969.54	5.47	65.8	↓	Response to oxidative stress; Response to wounding; Positive regulation of NF-kappaB transcription factor activity
7	RGD1311188 protein	B1WC62	40258.69	9.79	62.9	↓	Metal ion binding; Nucleic acid binding
9	LRRGT00008	Q6TXJ1	183308.21	10.44	65.6	↓	RNA binding
12	Bileacid-CoA: amino acid N-acyltransferase	Q63276	46777.17	7.46	101	↓	Organ regeneration; Liver development; Bile acid metabolism
13	Pink1	D3Z9M9	27675.61	11.4	71.2	↓	Cellular response to toxic substance; Positive regulation of I-kappa B kinase/NF-kappaB signaling; Response to stress
15	Proteasome subunit alpha type-6	P60901	27837.98	6.74	72.5	↓	Positive regulation of NF-kappaB transcription factor activity; Ubiquitin-dependent protein catabolic process
16	Proteasome subunit beta type-6	P28073	25515.54	4.61	147	↓	Proteolysis involved in cellular protein catabolic process
17	Protein Smarca5	F1LNL2	15813.06	9.65	63.1	↓	ATP-binding; DNA binding
20	Glyceraldehyde-3-phosphate dehydrogenase	P04797	122992.34	8.4	96.4	↓	Microtubule cytoskeleton organization
23	Apolipoprotein E	P02650	103861.79	4.93	64.2	↑	Negative regulation of inflammatory response; A ligand for the LDL (apo B/E) receptor
25	Itih4	Q5EBC0	35788.35	6.11	68.3	↑	Acute-phase response; Hyaluronan metabolic process
26	NmrA-like family domain-containing protein 1	P86172	34638.68	6.8	159	↑	Redox sensor protein; Reduces the production of nitric oxide
27	Ig kappa chain C region, B allele	P01835	26017.67	5.26	78	↑	Fc-gamma receptor signaling pathway involved in phagocytosis; Innate immune response
29	Ig lambda-2 chain C region	P20767	11481.67	5.8	70.5	↑	Immunoglobulin domain
30	Peroxisome oxidoreductin-2	P35704	21941.13	5.28	160	↑	Removal of superoxide radicals; Response to oxidative stress; Peroxidase activity
31	Galectin-9	P97840	36692.36	8.66	137	↑	Ion transport
32	Galectin-5	P47967	16414.11	6.66	91.7	↑	May function in erythrocyte differentiation
33	Galectin-5	P47967	16414.11	6.66	81.6	↑	May function in erythrocyte differentiation
34	Brefeldin A-inhibited guanine nucleotide-exchange protein 2	Q7TSU1	204296.96	6.48	68.1	↑	Golgi to plasma membrane transport; Intracellular signal transduction
36	Hbb-b1 hemoglobin, beta adult major chain	Q62669	16069.28	8.51	137	↑	Oxygen transport from the lung to the various peripheral tissues
37	Beta-2-microglobulin	P07151	13825.17	8.23	65.7	↑	Immune response; Response to drug
38	Hemoglobin subunit alpha-1/2	P01946	15489.83	8.14	373	↑	Oxygen transport from the lung to the various peripheral tissues
39	Ifi44l	M0R4J5	90125.05	8.97	69.5	↑	Immune response
40	Hemoglobin subunit alpha-1/2	P01946	15489.83	8.14	241	↑	Oxygen transport from the lung to the various peripheral tissues
41	C4-2 complement component 4, gene 2	Q6MG90	193602.9	7.04	255	↑	Inflammatory response; Complement activation
42	Complement C4	P08649	193638.91	7.34	202	↑	Inflammatory response; Complement activation; Innate immune response
43	Complement C4	P08649	193638.91	7.34	95.9	↑	Inflammatory response; Complement activation; Innate immune response

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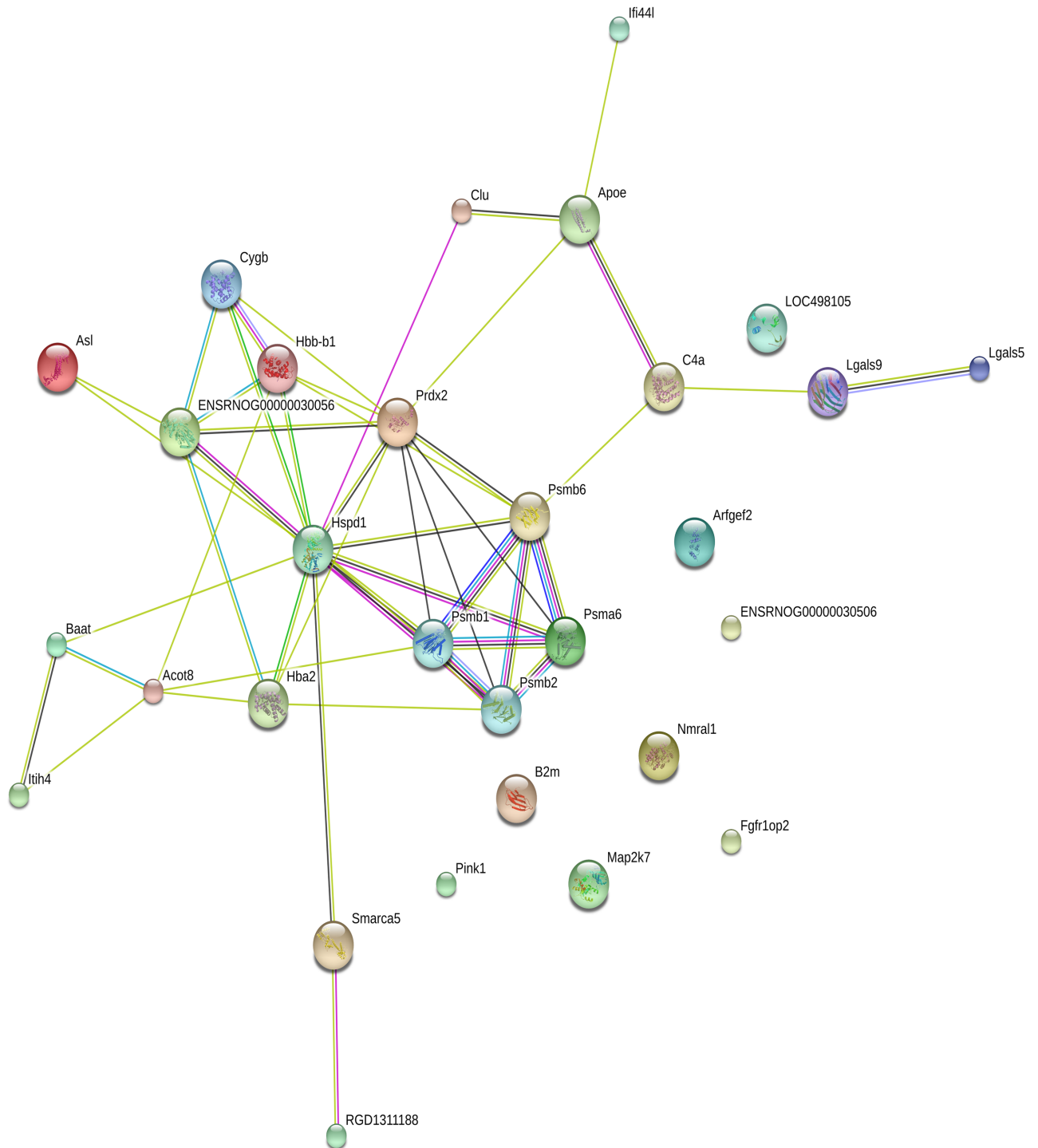


Fig 4. Analysis of Protein—Protein Interaction Network.

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group, and the livers of rats treated with rhCygb showed a reduction in liver injury, exhibiting a clear improvement. This suggests that rhCygb may play a role in a reversion of the progression of liver fibrosis, but the anti-fibrotic molecular mechanism remains under investigation. Experimental evidence and gene annotation showed that Cygb has many functions, including

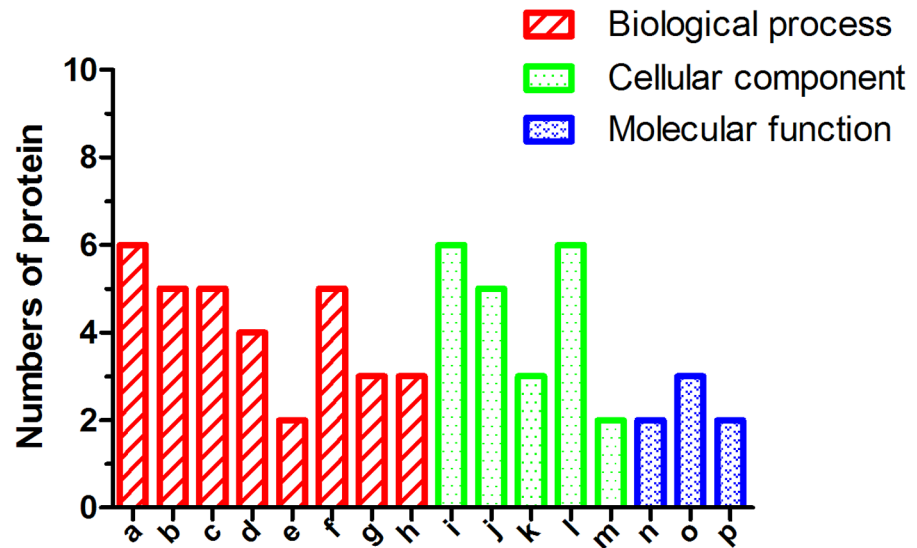


Fig 5. Distribution of all serum altered proteins into different functional and categories. a: response to oxidative stress; b: regulation of response to stimulus; c: regulation of immune system process; d: response to inflammatory; e: response to wounding; f: anti-apoptotic process; g: cellular macromolecule catabolic process; h: positive regulation of NF-κB transcription factor activity; i: extracellular region; j: cytosol; k: endosome; l: mitochondrion; m: plasome lipoprotein particle; n: ATP binding; o: ubiquitin protein ligase binding; p: antioxidant activity.

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O₂ storage and diffusion, which reduce oxidative stress, and sensing for cellular respiration and metabolism, NO scavenging and peroxidase activity, which protect cells against oxidative damage by free radical species. High-quality sample preparation prior to 2-DE is critical for producing meaningful results. To enhance detection of low-abundance serum proteins in 2-DE gels, we experimented with and compared the ProteoMiner kit[20] and the Albumin/IgG removal kit for removing the high-abundance proteins. As shown in Fig 2 and evidenced by the superior separation of analytes, protein enrichment and detection of low abundance proteins were far greater using the ProteoMiner kit than using the Albumin/IgG removal kit. Finally, we utilized the optimized conditions of 2-DE coupled with MALDI-TOF-MS analysis to obtain 43 spots and identify 30 proteins of interest.

Table 3. GO analysis of the corresponding differentially expressed proteins.

Protein function categories	Up-regulated in fibrosis model group	Up-regulated in rhCygb treatment group
Response to oxidative stress	Pink1; 60 kDa heat shock protein, mitochondrial	Apolipoprotein E; Peroxiredoxin-2; Hbb-b1 hemoglobin, beta adult major chain; Hemoglobin subunit alpha-1/2
Response to stimulus	Argininosuccinate lyase; FGFR1 oncogene partner 2 homolog	Peroxiredoxin-2; Beta-2-microglobulin; C4-2 complement component 4, gene 2
Regulation of immune system process		Complement C4; Beta-2-microglobulin; Ig lambda-2 chain c region; Ig kappa chain c region, B allele; Ifi441
Response to inflammation	60 kDa heat shock protein, mitochondrial	Apolipoprotein E; C4-2 complement component 4, gene 2; Complement C4
Oxygen transportation		Hemoglobin subunit alpha-1/2; Hbb-b1 hemoglobin, beta adult major chain; Galectin-5; Galectin-9
NF-κB signaling	Proteasome subunit beta type-6; Clusterin; Pink1	

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We analyzed the differentially expressed proteins and, taking into consideration their functional annotation, identified several proteins that may be related to the molecular mechanism of the anti-fibrotic effect of rhCygb treatment. Several proteins were down-regulated in the fibrosis model samples, yet were detected at high levels in the control and rhCygb treated groups. For example, hemoglobin subunit alpha -1/2 and hemoglobin subunit beta-1, which are directly involved in O₂ transport [6], and galectin-5 and galectin-9, which enhance O₂ transport by their participation in red blood cell differentiation, were under-expressed in the fibrosis model rats. Likewise, the anti-oxidant peroxiredoxin-2[23] was also found to be down-regulated in the fibrosis model samples. Proteins related to immune system processes, including Ig kappa chain c region, beta-2-microglobulin, Ifi441 and complement C4, were also found to be under-expressed in rats in the fibrosis model group. Previous reports have indicated that complement C4 could be a biomarker of liver fibrosis[24, 25]. Additionally, some proteins were found to be over-expressed in the fibrosis model group, including acute phase proteins such as 60 kDa heat shock protein (HSP60), FGFR1 oncogene partner 2 homolog and clusterin. Rats given chronic treatments of high concentrations of CCl₄ suffered liver tissue injury and necrosis. As part of the inflammation response, HSP60 was over-expressed to resist the adverse external stimulus. FGFR1 oncogene partner 2 homolog and clusterin, which are involved in the wound response[26], were similarly up-regulated. However, apolipoprotein E, which is a negative feedback inhibitor of the inflammatory response, was under-expressed in fibrosis model group. Among the proteins we identified and analyzed, proteasome subunit beta type-6, clusterin and pink1 which were over-expressed in the fibrosis model group are involved in NF- κ B signaling and are related to the initiation and regulation of immune and inflammatory responses, cell proliferation and tumorigenesis[27, 28]. The biological functions of the differentially expressed proteins relate mainly to oxidative stress[6, 29], immune and inflammatory responses, and NF- κ B signaling and give an insight into the underlying molecular mechanisms of the anti-fibrotic effect of rhCygb in the treatment of rats liver fibrosis. Given that liver biopsy is an invasive and expensive method, often poorly tolerated by patients, the use of easily obtained serum or plasma is a desirable choice for biomarker studies to improve the diagnosis of liver fibrosis patients through serum level detection.

Conclusion

In conclusion, the present study demonstrates that the effect of rhCygb on CCl₄-Induced hepatic fibrogenesis in rat. The rhCygb next study may be an original candidate drug in the treatment and reversal of liver fibrosis. However, further elucidation of the molecular roles played by the differentially expressed proteins of interest is necessary. Additionally, larger scale studies using human specimens will be required to further clarify the specific role of Cygb in hepatic fibrosis.

Author Contributions

Conceptualization: WD GC.

Data curation: BC.

Formal analysis: ZL PW.

Funding acquisition: WD.

Investigation: WW.

Methodology: GC.

Project administration: BC GC.

Resources: WD GC.

Software: GC BC.

Supervision: WD.

Validation: WD.

Visualization: GC.

Writing – original draft: WD.

Writing – review & editing: WD GC.

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