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Chapter 16

Can Proteomic Profiling Identify Biomarkers and/or Therapeutic Targets for Liver Fibrosis?

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/65608

Abstract

Liver fibrosis is a serious disease that affects around 350–400 million people worldwide. The main approach for fibrosis staging is liver biopsy, which is an invasive procedure that is not endured pretty well by patients. Currently, some serum-based biomarker panels are available for diagnosis and staging of liver fibrosis. Recent high-throughput proteomic studies are also very promising for identification of novel biomarkers for diagnosis and/or treatment of liver fibrosis. We hereby review the application of proteomic profiling studies for identification of fibrosis biomarkers with their advantages and drawbacks.

Keywords: proteome profiling, liver fibrosis, biomarkers, therapeutic markers

1. Liver fibrosis

Liver fibrosis results from chronic damage to the liver and causes accumulation of excessive matrix or scar. This scar tissue may inhibit blood flow due to the contraction of liver that results progressive liver damage and cirrhosis (the most advanced stage of liver fibrosis) or even hepatocellular carcinoma (HCC) [1]. Liver fibrosis is prominently observed in chronic liver diseases such as viral hepatitis, alcoholic steatohepatitis, nonalcoholic fatty liver disease (NAFLD), toxic liver injury, auto-immune diseases, and some genetic diseases [2]. From these chronic liver diseases, chronic hepatitis B (CHB) and chronic hepatitis C are major global health problems, and despite national vaccination programs, around 350–400 million people are infected with hepatitis B virus (HBV) and 130–150 million people are infected with hepatitis C

virus (HCV) worldwide [3, 4]. Chronic HBV (CHB) infection results in liver fibrosis that can further develop into cirrhosis or HCC, both being the major causes of liver-related death [5]. The annual incidence of cirrhosis in patients infected with HBV has been evaluated at 1.3–2.4% [6], and although the cumulative 5-year-old survival rate for patients with compensated cirrhosis is 84% [7], in patients with decompensated cirrhosis, this survival rate decreases to 14–35% [7, 8].

Regeneration of liver is an extremely complex process, but recent studies in human and animal models have indicated that liver fibrosis could be reversible in specific cases [9, 10]. It is hoped that deeper understanding of the etiology of liver fibrosis will contribute to improved diagnostic tools and potential therapeutic approaches for liver fibrosis and cirrhosis. Even though curing the underlying disease may reverse fibrosis progression, currently, the most effective treatment that prolongs survival in advanced cirrhotic patients is liver transplantation [11]. However, this approach is limited because of the shortages of organs, the presence of concurrent disease affecting other tissues, and recurrence of the original disease in transplant patients [12]. Despite the advancement in noninvasive tests, liver biopsy still remains as the gold standard test for evaluation of liver disease severity [13–16]. However, it has several disadvantages such as invasive character, sampling errors and limitations for effective surveillance, and follow-up [17–19]. Upon antiviral treatment, HCV-infected patients may clear HCV RNA from their bloodstream [5]. For the treatment of CHB, current therapies do not accomplish complete eradication of HBV infection. HBV remains in infected hepatocytes in the form of covalently closed circular DNA (cccDNA) even if the patient clears HBsAg, and this cccDNA can possibly be reactivated with the right stimulus [20]. Hence, the therapeutic strategy for CHB is to prevent liver fibrosis and the other complications of advanced liver disease that can further develop cirrhosis and HCC. Therefore, recent studies focus on the search of biomarkers for noninvasive diagnosis and staging of liver fibrosis and for discovery of new therapeutic targets to prevent HBV-related liver fibrosis.

Proteomics, which studies the complex protein mixtures in a biological system, is a valuable tool to investigate cellular pathways, protein–protein interactions, and identify target proteins [21]. No requirement of a priori knowledge of protein identities present in a biological system makes proteomic profiling an ideal tool for screening the most discerning set of biomarkers [22].

In this review, we will focus on the advances in the proteomic research concerning liver fibrosis and evaluate whether proteomic profiling studies are applicable in the search of protein biomarkers and/or therapeutic targets for this condition with a focus on HBV and HCV infection.

2. Pathogenesis and staging of liver fibrosis

Hepatic fibrosis develops as a result of wound healing response of the liver to chronic injury in conjunction with the deposition of extracellular matrix (ECM) proteins [23]. Deposition of ECM proteins forms a fibrous scar that alters hepatic architecture, and subsequent formation of nodules of regenerating hepatocytes results in cirrhosis [24]. After an acute liver damage

(e.g., HBV and HCV infection), parenchymal cells regenerate and substitute the necrotic and apoptotic cells. This process is accompanied with an inflammatory response and minor accumulation of ECM. Following persistent damage, eventually liver regeneration declines, and hepatocytes are replaced with abundant ECM, including fibrillar collagen. Origin of liver injury determines the distribution of this fibrous material. While in chronic hepatitis and chronic cholestatic disorders, the localization of fibrotic tissue is around portal tracts, in alcohol-induced liver diseases, its localization is in pericentral and perisinusoidal areas [25].

In the fibrotic liver, the main ECM-producing cells are hepatic stellate cells (HSCs) [26]. In the healthy liver, HSCs are found in the space of Disse and act as the major repository sites of vitamin A. Following sustained injury, HSCs activate or transdifferentiate into myofibroblast-like cells that have contractile, proinflammatory, and fibrogenic characteristics [27, 28]. Activated HSCs, which migrate and accumulate at the wound repair locations, secrete bulk amounts of ECM and mediate ECM degradation [29].

Some other hepatic cells, besides HSCs, may show fibrogenic properties. One of them is myofibroblasts derived from small portal vessels which reproduce around biliary tracts in cholestatis-induced liver fibrosis to induce collagen accumulation [30, 31]. The origin of the liver injury may determine the relative significance of each cell type in liver fibrogenesis. For instance, while HSCs exert the main fibrogenic activity in alcohol-induced liver fibrosis, portal myofibroblasts may be the most crucial fibrogenic cell types in viral hepatitis or chronic cholestatic disorders [1]. Thus, origin of liver injury may determine the molecular pathway differentiation in the formation of each liver disease, affecting the final proteomic outcome.

During fibrosis development, a complex interaction occurs between different hepatic cell types [32]. Most of the hepatoxic agents such as hepatitis viruses, alcohol metabolites, and bile acids target hepatocytes [33]. Injured hepatocytes secrete reactive oxygen species (ROS) and fibrogenic mediators, which triggers the activation of lymphocytes by inflammatory cells. Apoptosis of these injured hepatocytes further induces the fibrogenic actions of liver myofibroblasts [34]. Inflammatory cells such as lymphocytes and polymorphonuclear cells stimulate HSCs for collagen synthesis [35]. Activated HSCs also release inflammatory chemocines, secrete cell adhesion molecules, and mediate activation of lymphocytes [36]. Thus, a fierce cycle in which inflammatory and fibrogenic cells induce each other likely appears [37]. Kupffer cells, which are the local macrophages of liver, also greatly participate in liver inflammation by secreting ROS and cytokines [38, 39]. In conclusion, fibrogenesis is directly activated by alterations in the ECM composition and this altered ECM can serve as a repository for MMPs, growth factors, and inflammatory cytokines [1, 40].

Fibrosis progression is generally evaluated by two different accepted scoring systems: Ishak (modified Knodell score) and METAVIR scores. While in METAVIR, only interface hepatitis and lobular necrosis are used to determine the grade of activity, in Ishak, portal infiltrate and confluent necrosis are included with the two previous parameters [41]. Generally, fibrosis begins to develop as expansion of portal tracts occurring with interface hepatitis. As fibrosis advances, portal-portal linkage develops in conjunction with septa formation. At the end, fibrous tissue completely surrounds hepatocyte nodules. While complete cirrhosis develops generally in several years in some circumstances such as in the case of viral hepatitis, following

liver transplantation cirrhosis may develop much more rapidly. Parenchymal fibrosis can also be observed in the presence of lobular inflammation, especially in areas of bridging necrosis [42]. This may be the cause of portal-central septa formation, which has been considered as more crucial process in the development of cirrhosis than portal-portal linkages [43]. In the terminology of liver fibrosis, septa indicate expansion of portal tract edges without formation of bridges or actual connection between portal areas or portal area and central vein. On the other hand, the term bridge is used to assess actual fibrous connection between two portal areas or portal area and central vein [44]. It is important to consider these mentioned staging systems in a descriptive sense that a patient with stage 2 fibrosis cannot be assumed to have sustained twice as much liver damage as one with stage 1 fibrosis, nor half as much as one with stage 4 fibrosis because numerical stages are not evenly distributed along the progression of fibrosis, and also transition from one stage to the next one is not linear. Nonetheless, pathologists' interobserver agreement in fibrosis staging among one stage is approximately 90% [45, 46].

3. Biomarkers of liver fibrosis

An optimal biomarker of liver fibrosis would not get affected by functional distress in liver or kidneys and only be specific to liver, also be easily observed with simple, inexpensive, and noninvasive assays [13]. Liver enzymes that are routinely measured in serum such as alanine transaminase (ALT) and aspartate transaminase (AST) are not suitable biomarkers of liver fibrosis as they have poor correlation with liver fibrosis. Studies demonstrated that 20% of the biopsy-proven cirrhotic patients' ALT levels are in normal range [47]. Unfortunately, canonical markers of liver synthetic dysfunction [e.g., albumin, platelet count (PLT), prothrombin time (PT)] are shown to be unsuccessful in the detection of early fibrotic stages [48]. Currently, novel serum proteins have been observed with altered expression in progressing liver fibrosis such as apolipoprotein A1 (ApoA1), serum transferrin, and alpha 2 macroglobulin [49–51]. Biomarker panels that incorporate combination of these individual markers are also applicable for improved accuracy of fibrotic stage assessment [46]. The most currently used biomarker panels are AST to platelet ratio index [52], FibroTest that includes apolipoprotein A1 (ApoA1), haptoglobin (HPT), gamma-glutamyl transpeptidase (γGT), γ-globin, total bilirubin, and alanine aminotransferase as biomarkers [53], and FibroIndex that combines PLT, AST, and γGT [54]. These noninvasive biomarker panels have shown to achieve good negative predictive scores in patients with low fibrosis stages and good positive predictive scores in those with advanced stages. However, intermediate fibrotic stages are not successfully interpreted by these combined biomarkers [53]. Unfortunately, this setback limits the use of current available biomarker panels for routine clinical assessments of liver fibrosis [55].

4. Current proteomic profiling methodologies

Proteomics, which is a swiftly developing area, is currently preferred in discovery of novel disease biomarkers due to its potential to surpass the drawbacks of traditional screening

methods. The first step of the proteomic biomarker screening research is to separate and profile whole proteome of the biological fluid (e.g., serum, whole blood, saliva) or tissue of interest. Then, protein profile of the diseased sample is compared with a relevant control to identify the differentially expressed proteins related to that disease. Several different techniques based on in-gel separation and/or mass spectrometry are currently used for protein separation.

Mass spectrometry (MS) is the common technique in proteomic profiling methodologies. The basic concept of mass spectrometry is to evaluate the mass-to-charge (m/z) ratio for determination of the exact mass of the protein. The components of a mass spectrometry are an ion source, a mass analyzer, and a mass detector. Ionization of proteins is done either with matrix-assisted laser desorption/ionization (MALDI) or electrospray ionization (ESI). Following ionization, proteins pass through one or two mass analyzers that measure their m/z ratio (MS or versus tandem MS/MS). Time-of-flight (TOF) that measures the time spent by the protein through the vacuum tube in an electric field can be coupled with one or two quadrupoles (Q-TOF or Q-Q-TOF) with oscillating electric field that enables molecules with specific m/z ratios to travel without collision [56, 57].

4.1. Two-dimensional gel electrophoresis (2D-PAGE)

The 2D-PAGE technique separates protein according to two independent parameters, isoelectric point and molecular weight, and therefore provides the best resolution possible in protein separation currently [58, 59]. Following staining and digitalization with specific softwares, protein quantitation is performed by evaluation of spot intensities. 2D-PAGE also enables detection of posttranslational modifications, such as phosphorylation, or presence of different protein isoforms due to the emerging shifts in protein mass or isoelectric point [46]. In addition, two-dimensional difference gel electrophoresis (2D-DIGE) presents various advances including reproducibility, detection sensitivity, and credibility of analysis [60–62]. In 2D-DIGE, different samples are labeled with charge- and mass-matched fluorescent cyanine dyes, Cy3 and Cy5. The internal standard prepared by mixing equal amounts of all samples is labeled by Cy2. The Cy3 and Cy5 labeled samples and Cy2 labeled internal standard are then mixed and co-separated on the same 2-DE gel, providing accurate spot detection and intra-gel matching with reduced experimental variations. Running internal standard within all gels also improves gel-to-gel spot matching and enables for statistically strong comparisons between protein samples [63]. At the end, protein spots cut from 2D gels were identified by mass spectrometry [64].

4.2. Liquid chromatography coupled mass spectrometry (LC-MS)

Gel-based techniques such as 2D-PAGE are not very successful and reliable for profiling of small (>10 kDa) or hydrophobic proteins; besides, the evaluation of large numbers of samples is time-consuming and expensive. LC-MS, which couples a prefractionation stage with different types of mass spectrometry, is a relatively new gel-free proteomic methodology for proteomic profiling. One of the highly used MS methods is MALDI-TOF. In this technique, first, protein mixtures are fractionated by their physicochemical characteristics such as hydrophobicity or isoelectric point by liquid chromatography. Then, bound proteins are

vaporized and ionized by a laser. Finally, peptide mass is computed from the time spend to reach the detector ("time-of-flight"). Another frequently applied method is LC-MS/MS which efficiently profiles large numbers of samples with the analysis of extremely small volume samples (i.e., <75 μl) by evaluating proteins with masses ranging from 2 to 200 kDa with tremendous efficiency and reasonable reproducibility [65]. In addition, SELDI-TOF MS, which couples a prefractination stage with MALDI-TOF, is currently used for proteomic profiling studies. In SELDI, protein mixtures that selectively bind to an array with a specified characteristic are analyzed. This methodology requires very low amount of crude sample, such as serum or needle biopsy samples, and it is very efficient in analysis of low molecular weight proteins. Considering the minimal labor required for SELDI application, this technique is very useful for high-throughput screening. However, higher cost of SELDI still limits its large clinical scale usage [66–68].

5. Proteomic profiling studies in search of biomarkers for liver fibrosis

Proteomic studies on liver fibrosis mainly focus on cirrhosis and HCC, which are the very end and morbid stage of liver fibrosis. One of the earlier studies has compared tumor tissue and surrounding nontumor tissue from eight HCC patients and has showed overexpression of 14-3-3γ protein in HCC [69]. Another study has investigated the proteomic differences between tumor and adjacent nontumor tissue samples of 12 HBV-associated HCC patients and has found out upregulation of members of the heat shock protein 70 and 90 families and downregulation of metabolism-associated mitochondrial and peroxisomal proteins in HCC [70]. A recent study has analyzed sera of 40 HCC patients and 47 healthy controls and has discovered leucine-rich α2-glycoprotein (LRG) and haptoglobin (HPT) between HCV- and HBV-related HCC [71]. Molleken and Sitek (72) also have analyzed cirrhotic septa and liver parenchyma of seven cirrhotic patients and discovered an increase in cell structure-associated proteins, which are actin, prolyl 4-hydroxylase, tropomyosin, calponin, transgelin, and human microfibril-associated protein 4 (MFAP-4). However, all these studies investigate the alterations occurring at the very end stage of fibrosis and did not give information about the proteomic changes during fibrosis progression.

To identify therapeutic targets and their involved pathways in fibrosis, the proteomic changes between different fibrotic stages should be investigated. There are several studies that focus on proteomic changes between different fibrotic stages. One of these studies has investigated serum protein profiles of HCV-infected patients and has showed that Mac-2-binding protein, α-2-macroglobin, and hemopexin were increased in cirrhosis, and α-1-antitrypsin, LRG, and fetuin-A (also named as alpha-2-HS-glycoprotein) were decreased in cirrhosis [73]. A recent research, which has enrolled sera of 16 healthy controls and 45 HCV patients with different fibrotic stages graded due to METAVIR, has found out that α-2-macroglobin (A2M) was increased, while vitamin D-binding protein (VDBP) and apolipoprotein A1 (ApoA1) were decreased in late fibrosis [51]. One of the studies examining serum samples of seven healthy controls and 27 HBV-infected patients with different stages of fibrosis has shown that fibrinogen, collagen, A2M, hemopexin, α-1-antitrypsin, transthyretin, and

thiredoxin peroxidase were upregulated, while HPT, serotransferrin, CD5 antigen-like protein, clusterin, ApoA1, and LRG were downregulated along with fibrogenesis [74]. A recent study has analyzed sera of 19 CHB, six HBV-related cirrhotic patients, and five healthy controls and observed increased plasma myeloperoxidase levels in cirrhotic patients and decreased transthyretin, ceruloplasmin, and α-1-antitrypsin levels in both CHB- and HBV-related cirrhosis patients and downregulation of ApoA1 in HBV-related cirrhosis [75]. These studies about liver fibrosis have revealed the proteomic changes of serum samples throughout fibrogenesis. There are few studies that investigated proteomic changes in HCV-associated fibrogenesis. Diamond et al. demonstrated the effect of oxidative stress proteins to fibrosis progression in biopsy samples of HCV-infected patients [76]. The same group recently analyzed proteomic mechanisms of HCV-mediated liver fibrosis in posttransplant recipients by LC-MS (liquid chromatography coupled mass spectrometry) and demonstrated once again the important role of enhanced oxidative stress in the rapid fibrosis progression observed in HCV-infected liver transplant patients [77]. Ferrin et al. studied liver biopsies of HCV-infected alcoholic patients with cirrhosis for altered proteins in the progression of HCC and observed deregulation of ceruloplasmin (CP), paraoxanase (PON1), complement component 4a (CD4a), and fibrinogen-α (FGA) expression [78]. Another study investigated the differences in the protein profiles between liver samples from HBV-infected transgenic mouse and nontransgenic mouse and demonstrated increased aldehyde dehydrogenase 2 (ALDH2), protein disulfide isomerase precursor (PRDX1), actin, 78 kDa glucose-regulated protein (GRP78), tumor rejection antigen (GRP94), keratin 18 (KRT18), and decreased glutamate dehydrogenease 1 (GLUD1) and high mobility group 1 (HMGB1) protein levels [79]. An extensive list of potential biomarkers emerging from these studies is listed in **Table 1**.

Currently, studies also focused on understanding whether proteomic alterations may predict the treatment response in chronic hepatitis C. Hence, the effect of pegylated interferon (PegIFN) plus ribavirin (RBV) therapy, which is the common HCV treatment, may be understood better. When the serum samples from patients with chronic hepatitis C were subjected to metabolomics analysis to investigate the pretreatment and posttreatment characteristics of their metabolites by using capillary electrophoresis and liquid chromatography coupled mass spectrometry, tryptophan has been found to be associated with response to PegIFN/RBV therapy [82]. Moreover, identification of factors that predict virological response to antiviral therapy may improve treatment response through patient-specific treatment strategy. Recent studies revealed significant variances in proteome profiles throughout longitudinal serum samples in virological responders, in patients with mild fibrosis, and in those with mild necroinflammation [83]. In the current phase 2 studies (PROVE1, PROVE2, and PROVE3) of the direct-acting antiviral drug telaprevir, serum samples from responders and nonresponders to HCV treatment were analyzed by proteomic profiling and 15 differentially expressed proteins, with seven of them belonging to focal adhesion proteins or other macromolecular assemblies that constitute structural links between integrins and the actin cytoskeleton, were observed [84]. The ultimate goal of performing pretreatment serum proteome profiling prior to treatment is to predict sustained virological response (SVR) and nonresponse (NR) to antiviral drugs in chronic HCV infection and design suitable treatments for each patient.

Protein	Proteomic Analysis	Sample	Disease	Positive or Nagative Marker[a]	Reference
5′-3′ exoribonuclease 1	LC-MS	Liver biopsy	HCV	+	[77]
78 kDa glucose regulated protein, GRP78	2D-DIGE	Mouse liver tissue	HBV	+	[79]
A-1-antitrypsin	2D-PAGE	Serum	HCV	-	[73]
	2D-DIGE	Serum	HBV	+	[74]
Actin	2D-PAGE	Liver tissue	HCV	+	[72]
	2D-DIGE	Mouse liver tissue	HBV	+	[79]
Aldehyde dehydrogenase 2	2D-DIGE	Mouse liver tissue	HBV	+	[79]
Apolipoprotein A1	2D-PAGE	Serum	CHB	-	[75]
	2D-DIGE	Serum	HBV	-	[74]
	2D-DIGE	Serum	HCV	-	[51]
Aryl sulfotransferase 1A3	LC-MS	Liver biopsy	HCV	+	[77]
Bone martr stromal cell antigen 2	LC-MS	Liver biopsy	HCV	+	[77]
Calponin	LC-MS	Liver biopsy	HCV	+	[80]
	2D-PAGE	liver tissue	HCV	+	[72]
Carboxymethylenebutenolisade homologue	LC-MS	Liver biopsy	HCV	-	[77]
CD44 antigen	LC-MS	Liver biopsy	HCV	+	[77]
CD5 antigen like protein	2D-DIGE	Serum	HBV	-	[74]
Ceruloplasmin	2D-DIGE	Serum	HCV	+	[78]
Clusterin	2D-DIGE	Serum	HBV	-	[74]
Collagen	2D-DIGE	Serum	HBV	+	[74]
	LC-MS	Liver biopsy	HCV	+	[80]
Complement component 4a	2D-DIGE	Serum	HCV	+	[78]
Cystathione beta synthase	LC-MS	Liver biopsy	HCV	-	[77]
Cysteine and glycine rich protein 2	LC-MS	Liver biopsy	HCV	+	[80]
Cytochrome b-245 beta	LC-MS	Liver biopsy	HCV	+	[77]
Cytochrome c	LC-MS	Liver biopsy	HCV	+	[76]
Fetuin A	2D-PAGE	Serum	HCV	-	[73]
Fibrinogen	2D-DIGE	Serum	HCV	+	[78]
	2D-DIGE	Serum	HBV	+	[74]
Fibulin-5	LC-MS	Liver biopsy	HCV	+	[80]
FK506 binding protein 14	LC-MS	Liver biopsy	HCV	-	[77]
Gelsolin	2D-PAGE	Serum	HBV	-	[81]
Glutamate dehydrogenase 1	2D-DIGE	Mouse liver tissue	HBV	-	[79]
Gluthatione-S-transferases	LC-MS	Liver biopsy	HCV	-	[77]
Haptoglobin	2D-PAGE	Serum	HCV	-	[73]
	2D-DIGE	Serum	HBV	-	[74]
Hemopexin	2D-PAGE	Serum	HCV	+	[73]
	2D-DIGE	Serum	HBV	+	[74]
High mobility group 1	2D-DIGE	Mouse liver tissue	HBV	-	[79]

Protein	Proteomic Analysis	Sample	Disease	Positive or Nagative Marker[a]	Reference
Human leukocyte antigen class 2 antigen DR beta 1	LC-MS	Liver biopsy	HCV	+	[77]
Human leukocyte antigen class I antigen C	LC-MS	Liver biopsy	HCV	+	[77]
Keratin 18	2D-DIGE	Mouse liver tissue	HBV	+	[79]
Leucine-rich α-2-glycoprotein	2D-PAGE	Serum	HCV	-	[73]
	2D-DIGE	Serum	HBV	-	[74]
Leukotriene	LC-MS	Liver biopsy	HCV	+	[77]
Lumican	LC-MS	Liver biopsy	HCV	+	[80]
Mac-2-binding protein	2D-PAGE	Serum	HCV	+	[73]
Macroglobin	2D-PAGE	Serum	HCV	+	[73]
	2D-DIGE	Serum	HBV	+	[74]
	2D-DIGE	serum	HCV	+	[51]
Microfibril-associated glycoprotein 4	LC-MS	Liver biopsy	HCV	+	[80]
	2D-PAGE	liver tissue	HCV	+	[72]
Paraoxanase 1	2D-DIGE	Serum	HCV	+	[78]
Peroxiredoxin 1	2D-DIGE	Mouse liver tissue	HBV	+	[79]
Peroxiredoxin 2	2D-DIGE	Serum	HBV	-	[74]
Peroxiredoxin 5	LC-MS	Liver biopsy	HCV	+/-	[76]
Plasma myeloperoxidase	2D-PAGE	Serum	CHB	+	[75]
Prealbumin	2D-DIGE	Serum	HBV	+	[74]
Pre-angiotensionogen	LC-MS	Liver biopsy	HCV	+/-	[77]
Prolyl 4-hydroxylase	2D-PAGE	liver tissue	HCV	+	[72]
Protein disulfide isomerase precursor	2D-DIGE	Mouse liver tissue	HBV	+	[79]
Proteosome beta subunit type 4	LC-MS	Liver biopsy	HCV	+	[77]
Retinal dehydrogenase	LC-MS	Liver biopsy	HCV	+/-	[76]
Serotransferrin	2D-DIGE	Serum	HBV	-	[74]
Serum amyloid A1	LC-MS	Liver biopsy	HCV	+/-	[77]
Superoxide dismutase 1	LC-MS	Liver biopsy	HCV	-	[77]
Thioredoxin reductase 1	LC-MS	Liver biopsy	HCV	+	[77]
Transgelin	LC-MS	Liver biopsy	HCV	+	[80]
	2D-PAGE	liver tissue	HCV	+	[72]
Tropomyosin	2D-PAGE	liver tissue	HCV	+	[72]
Tumor rejection antigen, GRP94	2D-DIGE	Mouse liver tissue	HBV	+	[79]
Vitamin D binding protein	2D-DIGE	serum	HCV	-	[51]

[a] Proteins up- (+) or downregulated (S) in liver fibrosis, as detected in proteomic studies.
[b] When multiple comparisons have been performed between individual fibrosis stages certain proteins might have been reported as positive and negative markers.

Table 1. Candidate biomarkers of liver fibrosis identified from proteomic studies.

6. Limitations of proteomics

Proteomics have been shown as a promising tool in the evaluation of the molecular insights of liver fibrosis and in complementing previously known fibrosis biomarkers. Proteomic research is prone to unexpected and sometimes unpredictable biases [85]. Especially in analysis with multiple testing, extensive care should be given to assure that alterations observed are biologically significant and associated with the target disease [86]. Moreover, the unstable nature of biological samples makes them prone to degradation and alteration during sample processing [87]. Low-abundant proteins such as some stress expressed proteins and transcription factors are quite hard to be detected by proteomic screening.

Over 90% of the total serum protein concentration is constituted by some abundant proteins such as albumin and immunoglobins. Therefore, these abundant proteins may prevent detection of low-abundant proteins [88]. Depletion of serum from high-abundant proteins may increase the resolution and detection of low-abundance proteins [89]. However, while depleting serum from albumin, some potentially important proteins may bind to albumin and be lost for the upcoming analysis [90].

For the tissue samples, the diagnostic quality of biopsied tissue is limited for the evaluation of liver fibrosis. Presentation of only a very small part of the liver (approximately 1/50,000) by needle biopsy causes high sampling variability [91, 92]. Especially since fibrotic tissue is not distributed homogeneously inside the liver, sampling errors form 10% of false-negative diagnoses [91]. Moreover, interobserver agreement is not very high for particularly intermediate fibrosis stages. By considering these facts altogether, proteomic studies of liver fibrosis carry a robust characteristic.

7. Future directions and concluding remarks

Future studies in search of biomarkers for liver fibrosis should involve an adequate reference standard. Moreover, it is fairly possible that each chronic liver disease (CLD) could have its etiology-specific biomarkers, and further research should cover the identification of optimal biomarker sets for each cause of CLD (such as HBV, HCV, NASH, alcohol abuse). Serum proteomic studies might be combined with imaging techniques such as MALDI imaging to improve the performance of noninvasive techniques [93].

In summary, proteomic studies offer a great insight into differentially expressed proteins in plasma and hepatic tissue of patients with liver fibrosis. The results of this proteomic knowledge present researchers a better understanding about the pathobiology of liver fibrosis and lead to the discovery of the best set of biomarkers for the noninvasive assessment of the clinical stage of patients.

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