Review Article

Pathogenesis of hepatic fibrosis analyzed at the proteome level

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ABSTRACT

تقدم تقنية البروتينات الوراثية العديد من الطرق المؤثرة التي من شأنها تحليل أسباب مرض التليف الكبدي. لقد تم الكشف عن عدد كبير من البروتينات بفضل هذه التقنية، ومن شأن هذه البروتينات أن تلعب دوراً ملموساً في معرفة أسباب التليف الكبدي. ولقد أدى التقييم والفهم الشامل لهذه البروتينات إلى فهم ميكانيكية عمل مرض التليف الكبدي، وأن هذه الميكانيكية يمكن أن تُقسم إلى العديد من التصنيفات الواسعة. ونحن نقوم في هذا المقال بوصف ميكانيكية عمل المرض والتي يمكن أن ترتبط بكل من: القلق التأكسدي وضرر الانقسام الخيطي، والاستجابة للالتهاب وضرر جهاز المناعة، واضطراب حاصل في الانقسام والموت الخلوي، واضطراب في عمليات الأيض، واضطراب في التنبيغ الأحادي الخلوي، وأخيراً اضطراب في عمليات أيض المصفوفة خارج الخلية.

Proteomic technologies have provided effective approaches to the analysis of the pathogenesis of hepatic fibrosis. A large number of proteins that have been revealed by this technology play a critical role in various aspects of pathological liver fibrosis. Comprehensive evaluations of these proteins have led to the understanding that the mechanisms of hepatic fibrosis can be stratified into several broad classifications. Here, we describe the mechanisms of action that are defined as being related to 1) oxidative stress and mitochondrial damage, 2) inflammatory response and immune injury, 3) abnormal cell proliferation and apoptosis, 4) abnormal metabolism, 5) abnormal cellular signal transduction, and 6) abnormal extracellular matrix metabolism.

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Although the cellular mechanisms underlying hepatic fibrosis are only rudimentarily understood, the advent of proteomics has substantially improved the efficacy of liver fibrosis research. Proteomic technologies have advanced the understanding of the molecular mechanisms of liver fibrosis, led to the development of reliable biomarkers, and identified new drug targets. These advances have been the direct result of the identification of several differentially expressed proteins related to liver fibrosis. We performed a comprehensive review of the literature pertaining to the proteins involved in liver fibrosis or cirrhosis. We categorized these proteins based on their functional characteristics and found a wide range of reported biological functions, including cell cycle regulation, cytoskeleton development, cell proliferation and apoptosis, anti-oxidative stress, fatty acid oxidation, oxidative phosphorylation, lipid transport, protein synthesis and decomposition, glucose metabolism, immune regulation, inflammatory response, accumulation of extracellular matrix (ECM), signal transduction, molecular chaperones, and DNA repair and regulation. The purpose of this paper was to describe the pathogenesis of hepatic fibrosis based on the differentially expressed proteins broadly classified into functional categories (**Table 1**).

Oxidative stress and mitochondrial damage. Many types of liver injury will result in the production of free radicals, which will subsequently cause various degrees of oxidative stress. Long term exposure to increased levels of oxidative stress can lead to hepatic fibrosis and even cirrhosis. Proteomic studies revealed that the expression of the enzymes involved in the human antioxidant defense system was down-regulated when cirrhosis occurred. These enzymes included superoxide dismutase (SOD),[1-3] glutathione peroxidase (GPx),[1,3] and catalase.[3] The decreased generation of these enzymes may reduce the clearance and increase the production of reactive oxygen species (ROS). This can lead to a harmful accumulation of ROS, which will cause degeneration and necrosis of liver cell membranes, as well as subcellular organelles, through direct or indirect effects. This can propagate further hepatocyte damage, necrosis and apoptosis, leading to an eventual

Table 1 - Summary of differentially expressed proteins in tissues of liver cirrhosis from proteomic articles on hepatic disease.

Categories of pathogenesis	Differentially expressed proteins	Expression
Oxidative stress	SOD,[1-3] TRDP,[3-5] PRD6,[1,3] HO,[7] GPx,[1,3] GSTs,[7] ALDH,[7] STAP,[6] Catalase,[3] DJ-1 protein[2, 3]	-
Mitochondrial damage	ATP synthase-related proteins,[7] COX-related proteins,[7] UQCR-related proteins[7]	-
Immune injury	Haptoglobin,[8] AAG,[5] AMG [8]	-
Inflammatory response	HMGB1[9]	+
Abnormal cell proliferation and apoptosis	CDC27Hs,[4] CD23,[4] Gankyrin,[10] Cell cycle protein F,[10] PEBP,[2] ITIH4[1]	-
	p12,[11] Calcyclin,[12] Calgizzarin,[12] Galectin-1,[12] UBC7,[10] Caspase 12,[10] TTR,[8] IGFBP 2[2]	+
Abnormal protein metabolism	PSP[13]	+
	ER-60 protease,[13] Disulfide isomerase A2[4]	-
Abnormal lipid metabolism	Enoyl-coA hydratase,[13] HMGCS1,[1] ACAT,[1] PITP,[7] Apolipoprotein A1,[2,11] B100[8]/L1[14]/C1[7]	-
Abnormal ECM metabolism	HRG,[2] Human carboxypeptidase N[2]	-
	Angiotensinogen[8]	+
Differential expression of cytoskeleton proteins	β-actin,[8] Profilin1,[5] CK8[8]	+
Differential expression of heat shock proteins	HSP27,[14] HSP70,[7] HSP47[11]	+
Abnormal signal transduction	ISPK-1,[1, 7] PI3-K,[2,13] PKC,[13] 14-3-3 β protein,[13] lamin B1,[16] PTPs,[16] TGFβR I/ II/ III,[16] nPKCeta[17]	+
	TRAF1,[16,17] TRAF5[16, 17]	

Negative sign - downregulated expression in tissues of liver cirrhosis, positive sign - upregulated expression in tissues of liver cirrhosis, ACAT - Acetyl-coA acetyltransferase, ALDH - Aldehyde dehydrogenase, AMG - α2-macroglobulin, AAG - a-acidglycoprotein, CK8 - cytokeratin 8, COX-related proteins, Cytochrome C oxidase-related proteins, CD23 - cell division cycle protein 23, ER-60 protease, Endoplasmic reticulum (ER)-60 protease, GPx - glutathione peroxidase, GSTs - glutathione s-transferases, HSP27 - heat shock proteins 27, HMGCS1 - hydroxymethylglutaryl-coA synthase 1, HO - heme oxygenase, HRG - histidine-rich glycoprotein, HMGB1 - high-mobility group box 1 protein, IGFBP 2 - insulin-like growth factor binding protein2, ISPK-1 - insulin-stimulated protein kinase-1, ITIH4 - Inter-α-trypsin inhibitor-heavy chain 4, nPKCeta - epithelium-specific isoform of PKC, PEBP - phosphatidylethanolamine, binding protein, PKC - protein kinase C, PI3-K - phosphatidylinositol 3-kinase, PITP - phosphatidylinositol transfer protein, PRD6 - peroxiredoxin 6, PSP - perchloric acid-soluble protein, PTPs - protein tyrosine phosphatases, p12 - cyclin-dependent kinase inhibitor, SOD - superoxide dismutase, STAP - rat stellate cell activation-associated protein, TRDP - thioredoxin peroxidase, TTR - transthyretin, TRAF1 - TNF receptor-associated factor 1, TGFβR I - transforming growth factor-β type I receptor, TRAF5 - TNF receptor-associated factor 5, UBC7 - ubiquitin-conjugating enzymes, UQCR - related proteins, ubiquinol-cytochrome C reductase-related proteins.

inflammatory response in hepatic tissue, where a large number of inflammatory mediators and cytokines will be produced. As a result of this process, Kupffer cells can be activated, together with the inflammatory mediators and cytokines, these cells can activate hepatic stellate cells (HSC). Proteomic studies have also led to methods to counteract these deleterious changes via the discovery of several novel potential anti-oxidants including thioredoxin peroxidase (TDx),[3-5] DJ-1 protein,[2,3] peroxiredoxin 6 (PRD6),[1,3] rat stellate cell activation-associated protein (STAP),[6] glutathion s-transferases (GSTs),[7] aldehyde dehydrogenase (ALDH),[7] and heme oxygenase (HO).[7] These anti-oxidants can eliminate peroxides with high levels of antioxidative effect, which will also prevent macrophage oxidation. The down-regulated expression of a series of proteins related to oxidative phosphorylation may be attributed to the attack of ROS on mitochondrion. These down-regulated proteins included ATP synthase-related proteins,[7] cytochrome C,[7] cytochrome C oxidase-related proteins (COX-related proteins),[7] and ubiquinol-cytochrome C reductase-related proteins (UQCR-related proteins).[7] With an increased accumulation of ROS, lipid peroxidation will occur on the mitochondrial membranes and result in lower membrane fluidity, higher membrane permeability, and the eventual loss of the cytochrome. The mitochondrial changes will substantially impair mitochondrial oxidative phosphorylation, inhibit ATP generation and lead to complete cellular necrosis. This cellular death will accelerate the progression of liver fibrosis as a large number of cytokines will be released to the hepatic region.

Inflammatory response and immune injury. Another type of liver fibrosis-related protein discovered with proteomic technologies was categorized as immune regulatory factors and inflammatory factors. These proteins include the down-regulated haptoglobin,[8] α1-acidglycoprotein (AAG),[5] and α2-macroglobulin (AMG),[8] as well as the up-regulated high-mobility group box 1 protein (HMGB1).[9] The abnormal expression of this type of protein may result from hepatic parenchymal injury. These necrotic cells will be ingested by activated macrophages or megakaryocytes and inflammatory factors such as HMGB1 will be released to mediate the inflammatory response. During this process, rich α2-macroglobulins will be synthesized and enter into the

liver tissue, where they bind and thus eliminate excessive proteases. This defensive pathway prevents further damage to the liver tissue. The expression of the proteins involved in immune response, including haptoglobin and α1-acidglycoprotein (AAG), will be subsequently under-regulated to resist the inflammation. The whole process indicates that high level of inflammatory response will gradually lead to hepatic fibrosis.

Abnormal cell proliferation and apoptosis. The liver has the remarkable ability to regulate its own development and size. Although the hepatocytes of the adult liver rarely divide under normal physiological conditions, they possess the ability to proliferate following liver injury or the opposite when the liver has achieved an excessive size. The regeneration process of the liver includes a series of steps, 2 of which are critical for the ultimate propagation of the entire process. These 2 steps are: 1) a start signal must be generated and acts on the G0 cells to start the cell cycle and 2) the quiescent G0 cells must be driven into the G1 phase of the cell cycle. Proteomic studies demonstrated several proteins involved in cell cycle regulation were differentially expressed. CDC27Hs protein,[4] which regulates the mitotic phase of the cell cycle at the mid stage, cell division cycle protein 23 (CD23),[4] which regulates the transition of cells from the G2 phase to the M phase, Gankyrin,[10] and cell cycle protein F[10] were down-regulated. Contrary to these proteins, the expression of cyclin-dependent kinase inhibitor p12[11] was up-regulated. Taken together, these results suggest that the liver regeneration decreased. Additional studies of the HSC proteome demonstrated that the expressions of the proteins that regulate the growth and reproduction of HSCs, such as calcyclin, calgizzarin and galectin-1[12] were up-regulated.

The expression of ubiquitin-conjugating enzymes UBC7,[10] and caspase 12[10] were up-regulated, while the expression of the serine proteinase inhibitor, phosphatidylethanolamine-binding protein (PEBP)[2] was down-regulated, both of which are involved in apoptosis. The results from these studies indicated that some type of hepatic cell was undergoing apoptosis as cirrhosis progressed. However, the expression of both transthyretin (TTR),[8] a negative acute-phase protein, and insulin-like growth factor binding protein 2 (IGFBP 2),[2] both of which are related to liver cell repair, growth and differentiation, were up-regulated. The expression of inter-α-trypsin inhibitor-heavy chain 4,[1] which can promote the regeneration of liver, was down-regulated. These findings suggest that liver regeneration may be limited due to serious damage during the later stages of cirrhosis.

Abnormal metabolism. *a) Abnormal protein metabolism.* The studies with proteomic technologies discovered that the expression of perchloric acid-soluble protein[13] which inhibits protein synthesis was up-regulated. The expression of endoplasmic reticulum (ER)-60 protease[13] contributing to the degradation of abnormal proteins, and protein disulfide isomerase A2[4] contributing to normal protein folding was down-regulated. These indicated that protein synthesis was inhibited and abnormal proteins were accumulated in cells, leading to the loss of normal protein function, and ultimately the loss of cell function and cell necrosis, which promoted the fibrosis progression

b) Abnormal lipid metabolism. The expressions of most enzymes involved in fatty acid oxidation were down-regulated. These enzymes included enoyl-coA hydratase,[13] hydroxymethylglutaryl-coA synthase1 (HMGCS1),[14] and acetyl-coA acetyltransferase (ACAT).[6] The down-regulation of these proteins indicated a reduction in fatty acid oxidation. Reduced fatty acid oxidation will result in the accumulation of lipids and the secretion of a large number of cytokines and adipokines. Up-regulation of collagen will occur and cell proliferation will be stimulated. This will lead to the subsequent apoptosis of hepatocytes, as well as the up-regulation of proinflammatory cytokines and proangiogenic factors. The synthesis of the ECM will increase, leading to cirrhosis of the liver.The expression of lipid-transfer proteins including apolipoprotein A1,[2,11] apolipoprotein B100,[8] apolipoprotein L1,[15] apolipoprotein C1,[7] and phosphatidylinositol transfer protein[7] was down-regulated. This down-regulation suggests that lipid transport was inhibited in liver blood circulation, resulting in the accumulation of lipids within liver, which will further contribute to fibrotic changes and cirrhosis.

Abnormal ECM metabolism. The use of proteomic technologies revealed that the expression of histidine-rich glycoprotein (HRG)[2] and human carboxypeptidase N[2] was down-regulated. The expression of angiotensinogen,[8] the angiotensin precursor, was up-regulated. These proteins are closely related to ECM metabolism, in particular histidine-rich glycoprotein and human carboxypeptidase N regulate the activation of plasmin. The reduced expression of these proteins will decrease the levels of plasmin activity, result in the inactivation of bradykinin and reduce the activity of vasodilation. The down-regulation of these proteins along with the up-regulated expression of the angiotensin precursor, angiotensinogen,[8] will increase the synthesis of ECM. Alteration in the composition of the ECM during liver cirrhosis mainly includes the down-regulated expression of non-collagen glycoprotein and fibronectin.

Mechanisms of differential expression of cytoskeleton proteins and heat shock proteins. Microfilaments composed of actin participate in various cellular

activities that are critical to function on both the cellular and macro-cellular levels. These functions include maintaining cellular morphology, muscle contractions, amoeboid cell movement, cell division, cell migration and signal transduction. These activities also play crucial roles in the development and progression of liver fibrosis. Therefore, the differential expression of cytoskeleton proteins can substantially impact hepatic fibrosis progression. Proteomic studies have shown that the cytoskeleton proteins including β-actin,[8] Profilin[16] and cytokeratin[8] (CK8)[6] demonstrated differential expression during hepatic fibrosis.

Heat shock proteins (HSP) play important roles in the intracellular homeostasis, particularly in cellular tolerance to stressors and synergistic actions of immune complexes. Proteomic technologies have also led to the realization that many members of the heat shock protein family, including HSP27,[15] HSP70,[7] and HSP47[11] showed differential expression during liver fibrosis. As a result of the differential responses, the mechanisms of action have been described as follows: when stressors induce immune responses, HSPs are produced along with various kinds of cytokines, to resist the damage of cytokines on liver tissue and cells.

Abnormal signal transduction. Many proteins related to signal transduction are differentially expressed in cases of liver fibrosis. The expressions of 1) protein kinases including insulin-stimulated protein kinase-1 (ISPK-1),[1,14] phosphatidylinositol 3-kinase (PI3-K),[13,2] and protein kinase C (PKC),[13] 2) regulatory proteins, including protein kinase C-β inhibitor 14-3-3 β-protein,[13] PKC activator nuclear laminar protein lamin B1[17] and protein tyrosine phosphatases (PTPs),[14] 3) cytokine receptors, including transforming growth factor-β type I receptor (TGFβR I),[16] transforming growth factor-β type II receptor,[14] and transforming growth factor-β type III receptor,[14] and 4) the epidermal growth factor-binding protein, epithelium-specific isoform of PKC (nPKCeta),[17] were up-regulated. The expressions of tumor necrosis factor (TNF) receptor-associated proteins regulating apoptosis, such as TNF receptor-associated factor 1 (TRAF1),[14,17] and TNF receptor-associated factor 5 (TRAF5)[14,17] were down-regulated.

In conclusion, the process of hepatic fibrosis is complex and includes multiple pathways. The mechanisms mentioned above pertaining to liver fibrosis are all correlated, and in some cases, reliant on one another. Future studies are required to examine the particular interactions between the proteins involved in liver fibrosis. The results from proteomics studies in liver disease have substantially contributed to understanding of the disease process. Future work in this field will reveal additional novel proteins that may aid in targeted therapeutics or molecular biomarkers. Future studies should evaluate the structure and function of these proteins to advance the knowledge of mechanisms underlying liver fibrosis and lead to eventual effective therapeutic interventions.

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